Effect of 13-cis-Retinoic Acid and α-Interferon on Transforming Growth Factor β1 in Patients with Rising Prostate-specific Antigen

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ABSTRACT

The objective of this study was to test the hypothesis that 13-cis-retinoic acid (CRA) and α-interferon (IFN-α) have antitumor activity in patients with early recurrence of prostate cancer measured by rising prostate-specific antigen (PSA) after local therapy, and that this activity is associated with the increase of plasma transforming growth factor β1 (TGF-β1).

Thirty patients with a PSA > 7 ng/ml that increased >0.4 ng/ml/month after initial radiation therapy or a PSA > 2.0 ng/ml after prostatectomy were treated with 1 mg/kg/day of CRA and 3 million units of IFN-α administered three times per week. Patients were followed clinically with serum measurements of PSA and assessment of toxicity. Biological activity of CRA and IFN-α was assessed by the measurement of plasma TGF-β1.

Twenty-six percent of patients had a partial (50% decrease maintained for 1 month) or minimal (<50% decrease maintained for 1 month) biochemical response of PSA, with a median decrease of 23% (11–55%) at 3 months. Plasma TGF-β1 levels increased with CRA and IFN-α therapy and correlated with a decrease in PSA; patients with a decrease in PSA had a 151% increase in TGF-β1 compared to 27% in patients without a decrease in PSA (P = 0.04).

CRA and IFN-α can produce transient reduction or stabilization of PSA. The measurement of plasma TGF-β1 at 1 month of therapy correlates with changes in PSA and may represent a useful marker for the biological effect of these agents; further analysis in larger numbers of patients and methods to optimize these effects should be explored.

INTRODUCTION

The early detection and treatment of recurrent malignancies offers the theoretical advantage of being able to improve outcome. However, few malignancies produce sufficiently sensitive markers to test this hypothesis; cancer of the prostate is one exception. PSA elevation after initial local therapy for prostate cancer predicts disease progression. Because the prostate is the only source of PSA, any detection of PSA after prostatectomy represents persistent prostate cancer and a high rate of disease recurrence (1, 2). Patients with rising PSA after radiation therapy for localized prostate cancer similarly represent a group of patients with a high rate of disease recurrence (3–5).

Although the elevation of PSA after local therapy for prostate cancer is indicative of early disease progression, effective therapy for these patients is limited. Androgen ablation produces only temporary responses; the recurrent disease is then highly resistant to treatment (6–8). Biological therapies offer a new approach to the treatment of early recurrent prostate cancer and may not be affected by known drug-resistance mechanisms. For example, retinoids inhibit growth and induce apoptosis in both hormonally sensitive and resistant prostate tumor cells (9, 10). Additionally, animal studies have shown that the benefit of retinoids may be greatest when disease is minimal (11). IFN-α and CRA have synergistic activity against prostate cancer cell lines, and the combination has some clinical activity in other tumors (12, 13). Therefore, IFN-α may improve the antitumor activity of CRA in patients with less volume of disease.

A major difficulty in assessing the appropriate use of biological therapies in early recurrent disease, however, is the lack of a verified indicator of therapeutic activity; an accurate measure of subclinical biological activity could be used to optimize therapy in future studies. The use of PSA alone as an indicator of treatment effect may not be reliable. For example, evaluation of the prognostic significance of a single posttherapy PSA in patients treated with suramin and hydrocortisone...
showed no association between a given degree of decline and outcome (14). Additionally, some patients treated with CRA and IFN-α demonstrated an initial increase before a decrease in PSA (15).

The induction of TGF-β1 may be a useful indicator of a biological response to retinoids. Retinoids are potent inducers of TGF-β. TGF-β1 is an inhibitory growth factor that belongs to a family that consists of three isoforms (TGF-β1, -2, and -3) and inhibits the growth of epithelial cells (16). For example, TGF-β inhibited the proliferation of DU-145 and PC-3 cells (both hormone-resistant prostate tumor cell lines), and the addition of IFN-α was additive (16). Therefore, TGF-β may be a useful marker for CRA and IFN-α action in patients with early prostate cancer and may help to define the use of PSA as a surrogate marker.

We now report the results of our clinical trial of CRA and IFN-α in patients with early biochemical recurrence of prostate cancer after local therapy. We evaluated whether plasma TGF-β1 was useful in predicting the clinical activity of these biological agents.

PATIENTS AND METHODS

Patient Eligibility. Patients were required to have histologically proven prostate cancer and to have completed initial local therapy (radical prostatectomy or definitive radiation therapy). In patients with prior prostatectomy, PSA must have been >2 ng/ml by two measurements at least 1 month apart. In patients with prior radiation therapy, PSA must have been >7 ng/ml with a rate of increase (PSA velocity) greater than 0.4 ng/ml/month, determined by three values, each at least 1 month apart. Patients with measurable disease recurrence were not eligible for this trial.

Requirements for inclusion included: (a) Eastern Cooperative Oncology Group performance status of ≤2; (b) adequate blood counts (WBC of ≥3,500/μl and platelet count of ≥100,000/μl); (c) adequate liver function studies (aspartate aminotransferase and alanine aminotransferase ≤2 times normal, and bilirubin ≤1.5 mg/dl); and (d) adequate renal function (creatinine ≤1.5 mg/dl).

For patients receiving hormonal therapy, the therapy was continued to avoid the confounding effects of hormonal withdrawal on PSA. All patients were required to give written informed consent, which was approved by the Institutional Review Board at the Robert Wood Johnson Medical School.

Treatment Plan. All patients received 1 mg/kg/day of CRA p.o. and 3 million units of IFN-α s.c. three times each week (Acutane and Roferon were provided by Roche Pharmaceuticals, Nutley, New Jersey). One cycle of therapy was 28 days; PSA measurement was obtained with each cycle. An assessment of PSA change was made every three cycles, and therapy was discontinued for biochemical progression (defined as ≥25% increase in PSA over baseline) or unacceptable toxicities (National Cancer Institute grade 3 or 4 toxicity). Patients with grade 2 hypertriglyceridemia from CRA were treated with Lopid.

Study Outcome Variables. Pretreatment evaluation of patients included a bone scan, chest radiograph, and laboratory studies, including PSA and plasma TGF-β1. Laboratory studies, including TGF-β1, were obtained each cycle (every 28 days).

We defined PSA biochemical response as follows: (a) decreases in PSA were called biochemical responses to differentiate them from the standard clinical responses; (b) partial biochemical response was defined as a ≥50% decrease in PSA lasting ≥1 month; and (c) minimal biochemical response was defined as a reduction in PSA of <50% lasting ≥1 month.

Laboratory Studies. TGF-β1 was analyzed in plasma obtained by collection of 5 ml of blood in EDTA vacuum tubes. The blood was centrifuged immediately at 1500 rpm for 20 min. The plasma was removed with care not to disrupt the buffy coat to avoid TGF-β1 contamination from platelets. The plasma was stored at −70°C until assayed for TGF-β1.

Extraction of total TGF-β1 was performed by a modification of the acid/ethanol procedure of Roberts et al. (17) as described previously (18). The ELISA used for measuring plasma TGF-β1 is a sandwich assay using the monoclonal antibodies 12H5 and 4A11 provided by Genentech (South San Francisco, CA; Ref. 18). Horseradish peroxidase-conjugated rabbit antimouse IgG-1 was applied as the secondary antibody. The amount of TGF-β1 in the patient samples was determined by comparing the peroxidase activity in wells containing known quantities of purified TGF-β1 (R&D Systems, Minneapolis, MN). The procedure measures total TGF-β1 (18).

Statistical analysis was conducted using a Spearman correlation coefficient for correlation analysis comparing PSA effect and TGF-β1 increase. PSA effects in patients with an increase of TGF-β1 and without an increase of TGF-β1 were compared by an ANOVA model in which we adjusted for the percentage changes from baseline in months 1, 2, and 3.

RESULTS

Thirty patients were entered onto this protocol (Table 1). The median age of patients was 68 years (range, 55–82 years). All patients had prior radiation therapy (n = 10) or prostatectomy (n = 20) as initial primary therapy for localized prostate cancer. Nine patients had progressed after androgen ablation, and 21 had no prior hormonal therapy. Patients that progressed on androgen ablation therapy were continued on that therapy during treatment with CRA/IFN-α to avoid the confounding effects of a hormonal withdrawal response. Twenty-seven of the initial 30 patients completed at least 3 months of therapy. Two patients did not complete 3 months of therapy secondary to the development of unrelated medical problems (one developed congestive heart failure, and one had a severe trauma, with...
Fig. 1 Change in plasma TGF-β1 during CRA and IFN-α therapy (represented as the mean percentage increase above the baseline). Patients included had a baseline value and a plasma level at 1 (n = 18), 2 (n = 22), or 3 (n = 20) months. TGF-β1 was elevated during the 3 months of therapy, with a mean increase of 58% at 1 month (SE = 34; P = 0.11).

Fig. 2 TGF-β1 change (mean percentage increase) from baseline was compared in patients with a decrease in PSA at 3 months and patients with an increase in PSA at 3 months. □, patients with a decrease in PSA during therapy; ■, patients with an increase in PSA during therapy. Monthly plasma TGF-β1 levels obtained with a baseline value with which to compare are represented. Patients with a decrease in PSA at 3 months have TGF-β1 levels represented as a percentage increase compared to baseline by a bar at 1 (n = 5), 2 (n = 6), and 3 (n = 7) months. Patients with an increase in PSA at 3 months also have TGF-β1 levels represented as a percentage increase compared to baseline by a bar at 1 (n = 12), 2 (n = 14), and 3 (n = 13) months. Patients with decreased PSA (n = 5) had a significantly greater increase in TGF-β1 after 1 month compared to patients with an increase in PSA (n = 12; 150.9% increase versus 26.9% increase; P = 0.04). Tables 2 and 3 provide the actual values for TGF-β1 in patients with a decrease in PSA (Table 2) and patients with an increase (Table 3) in PSA.

discontinuation of therapy). One patient did not have a recent baseline PSA with which to compare values.

At 3 months of therapy, 7 of 27 patients (26%) had a partial (1 patient) or minimal (6 patients) biochemical response in PSA with a median decrease of 23% (11–55%). Overall, the median duration without progression of PSA (>25% increase) on therapy was 3.0 months (range, 2–9 months). Of the seven patients with biochemical response in PSA, two had hormone-refractory disease, and five had hormone-sensitive disease. The one patient with a partial response in PSA (a decrease of 55%) had originally progressed after androgen ablation.

TGF-β1 in the plasma was successfully measured over 3 months of therapy with CRA and IFN-α in most patients (Fig. 1). Fig. 2 demonstrates that the increase in TGF-β1 noted after 1 month of CRA and IFN-α therapy correlated to a decrease in PSA at 3 months, because patients with a decrease in PSA (n = 5) had a greater increase of TGF-β1, in contrast to patients without any decrease in PSA (n = 12; P = 0.04). Tables 2 and
2002 Effect of CRA and IFN-α on TGF-β1

Table 2 Serum PSA and plasma TGF-β1 values (ng/ml) for patients treated with CRA and IFN with a decrease in PSA at 3 months

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*NA, values that were not able to be obtained.

Table 3 Serum PSA and plasma TGF-β1 values (ng/ml) for patients treated with CRA and IFN with an increase in PSA at 3 months

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*NA, values that were not able to be obtained.

3 show the actual TGF-β values in the group of patients that had a decrease in PSA at 3 months (Table 2) and in the group of patients that had an increase in PSA at 3 months (Table 3).

The toxicities of therapy with CRA and IFN-α were minimal. Twenty-eight of 30 patients had grade 1, and 2 patients had grade 2 dry skin. Fourteen of 30 patients had grade 1, and 1 patient had grade 2 fatigue. Three patients had grade 2 hypertriglyceridemia, and two required Lopid therapy while on study. Although no grade 3 or 4 toxicity was noted to definitely be related to therapy, one patient developed MG while on therapy and later required ventilatory support and died from a septic event.

**DISCUSSION**

The combination of CRA and IFN-α has demonstrated antitumor activity in some advanced malignancies such as cervical cancer and squamous cancer of the skin (12, 13). Preliminary studies of CRA and IFN-α in prostate cancer also demonstrate some antitumor activity (15, 19). Higano and Chiellens (19) evaluated the use of CRA and IFN-α in patients with hormone-refractory prostate cancer and reported that some patients had a decline of PSA; PSA decreased to some degree in five of six patients, and one patient experienced a 73% decrease.

Kelly et al. (15) also reported the results of a Phase II trial of CRA and IFN-α in patients with prior hormonal therapy. They noted that one patient had a decline of PSA of greater than 50% and noted a >50% decline in PSA after discontinuation of therapy in an additional patient.

In contrast to these trials using CRA/IFN-α in patients with hormone-refractory metastatic prostate cancer, our study evaluated the effect of CRA/IFN-α predominantly in a group of patients with only biochemical progression, and presumably less volume of disease, before use of ablative therapy (21 of 30 patients). This clinical setting may be more relevant, because animal studies have suggested that the greatest activity of retinoids is against minimal disease (11), and because prior androgen ablation may induce resistance mechanisms, such as the expression of bcl-2, in contrast to hormone-sensitive tumors that have low levels of bcl-2 (6, 7, 8). However, because prior androgens induce cell death in androgen-independent cell lines, we predicted equivalent activity in patients with androgen-independent and androgen-sensitive tumors. In our trial, the numbers of patients in each of these groups (only 9 of 30 with prior hormonal therapy) is too small to determine whether TGF-β1 levels increased and PSA levels decreased equally in both populations. One example, however, that hormonal resistance...
did not effect CRA/IFN-α activity was a patient who had a 55% decrease in PSA with CRA/IFN-α therapy, despite initial progression after hormonal therapy.

Our trial, similar to these trials using CRA/IFN-α in prostate cancer, also noted minimal activity in the reduction of PSA. However, there are no data that validate the magnitude of PSA drop that confers an impact on clinical outcome; thus a <50% decrease may be important. Whereas several investigators previously suggested that posttherapy PSA declines can be used as a surrogate end point to evaluate new agents in hormone-refractory prostate cancer, other studies have demonstrated the lack of correlation of PSA to tumor effect (14). For example, Sridhara et al. (14) evaluated the data from 103 patients with hormone-refractory prostate cancer treated with suramin and found no significant overall survival differences between groups of patients with and without PSA decreases of ≥50 or ≥75%, suggesting that PSA reduction could not be used as a reliable response criterion to evaluate treatment efficacy.

In our study, plasma TGF-β1 was also evaluated as a marker of biological activity before and during therapy with CRA/IFN-α. Prior measurements of TGF-β consisted of immunohistochemical staining of tumor biopsy samples. This approach has the inherent limitations of specimen acquisition before and during treatment and nonquantitative determinations. A new approach to this problem is the measurement of plasma levels of TGF-β, because current data indicate that plasma levels are quantitative, are elevated in a variety of tumors (including hepatocellular carcinoma, prostate carcinoma, and breast cancer), and correlate with tumor volume (18, 20). Additionally, the induction of plasma TGF-β2 has been demonstrated with other agents that induce tissue TGF-β such as tamoxifen (21, 22).

We found an increase of plasma TGF-β1 during therapy, implying that a biological effect was induced in some patients. We also found that patients with a decrease in PSA had significantly greater elevation of plasma TGF-β1 levels at 1 month. We felt that the 1 month value of TGF-β1 was the most relevant, because drug effects should occur early, and later measurements could be altered by any increase or decrease in tumor volume (18). Additionally, prior studies evaluating plasma TGF-β2 levels in patients with breast cancer treated with tamoxifen demonstrated maximum levels of TGF-β2 at 1 month (21). Kopp et al. (21) evaluated plasma TGF-β2 levels in 20 patients with metastatic disease treated with tamoxifen and found that the induction of TGF-β2, which occurred predominantly at 1 month, correlated with clinical response to 3 months of tamoxifen therapy. Because the 1 month TGF-β1 value may be most relevant, based on this prior literature, we compared the TGF-β1 values at 1 month in patients with a decrease in PSA and patients without any effect on PSA. Our study also demonstrated that an increase in TGF-β1 during CRA/IFN-α at 1 month correlated with biochemical PSA response, although our numbers are small, and studies with larger numbers of patients need to be completed to confirm these findings.

The toxicity of the CRA/IFN-α combination is generally minimal, with symptoms such as dry skin, fever, and other flu-like symptoms, as was seen in our study (12, 13). However, one patient in our trial also developed MG and succumbed to sepsis. A review of the literature on MG and CRA and/or IFN-α reveals two reported cases of transient MG with elevation of acetylcholine receptor antibody in association with therapy with IFN-α (23). In both cases, the MG resolved with the discontinuation of IFN-α (23). Although this seems to be a rare occurrence, IFN-α, perhaps potentiated with CRA, might induce MG in susceptible individuals and must be considered a serious potential complication of this therapy.

Our data, therefore, show that CRA/IFN-α produces an effect on PSA and plasma TGF-β1. We have demonstrated that CRA/IFN-α can increase the levels of plasma TGF-β1 and give preliminary data suggesting a correlation of induction of TGF-β1 to decreased PSA. However, a larger trial in a more homogeneous group of patients would be required to confirm whether CRA and IFN-α treatment has any efficacy in reducing PSA values in patients with prostate cancer or increasing TGF-β plasma levels.

REFERENCES


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