Inhibition of NF-κB by (*E*)3-[(4-methylphenyl)-sulfonyl]-2-propenenitrile (BAY11-7082; BAY) is associated with enhanced 12-*O*-tetradecanoylphorbol-13-acetate-induced growth suppression and apoptosis in human prostate cancer PC-3 cells

XI ZHENG[1], RICHARD L. CHANG[1], XIAO-XING CUI[1], GINA AVILA[1], MOU-TUAN HUANG[1], YUE LIU[1], AH NG TONY KONG[2], ARNOLD B. RABSON[3,4] and ALLAN H. CONNEY[1,3]

[1]Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology; [2]Department of Pharmaceutics, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854; [3]Cancer Institute of New Jersey, New Brunswick, NJ 08903; [4]Center for Advanced Biotechnology and Medicine and the University of Medicine and Dentistry of New Jersey - Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA

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Abstract. The effects of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) alone or in combination with an NF-κB inhibitor, (*E*)3-[(4-methylphenyl)-sulfonyl]-2-propenenitrile (BAY 11-7082; BAY), on the growth and apoptosis of human prostate cancer PC-3 cells cultured *in vitro* or grown in immunodeficient mice were studied. Treatment of cultured PC-3 cells with TPA (0.2-10 ng/ml) for 96 h resulted in growth inhibition and apoptosis in a concentration-dependent manner. BAY inhibited NF-κB activity in PC-3 cells as determined by a luciferase reporter assay and enhanced TPA-induced growth inhibition and apoptosis in cultured PC-3 cells. In animal studies, NCr immunodeficient mice were injected subcutaneously with PC-3 cells in Matrigel. Mice with well-established tumors received daily i.p. injections with TPA (100 ng/g body weight/day), BAY (4 μg/g/day), or a combination of TPA (100 ng/g/day) and BAY (4 μg/g/day) for 36 days. Tumor growth occurred in all of the vehicle-treated control mice. The percent of animals with some tumor regression after 36 days of treatment was 0% for the control group, 40% for the TPA group, 50% for the BAY group and 100% for the TPA + BAY group. Mechanistic studies indicated that treatment of the mice with TPA or TPA + BAY decreased proliferation and increased apoptosis in the tumors. Results from our studies indicate that inhibition of NF-κB activity is associated with enhanced TPA-induced growth inhibition and apoptosis in PC-3 cells. Inhibition of NF-κB activity by suitable pharmacological inhibitors may be an effective strategy for improving the therapeutic efficacy of TPA in prostate cancer.

Correspondence to: Dr Allan H. Conney, Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020, USA
E-mail: aconney@rci.rutgers.edu

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Introduction

The phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), is the major active constituent in the seed oil (croton oil) obtained from the leafy plant Croton *tiglium* L of the Euphorbiaceae family. TPA is a potent stimulator of differentiation and an inhibitor of proliferation in myeloid leukemia cells (1,2). In a preliminary study, our laboratory together with colleagues in China demonstrated beneficial effects from intravenously administrated TPA for the treatment of seriously ill myeloid leukemia patients refractory to other therapy. Decreased number of myeloblasts in the bone marrow and in the peripheral blood was observed as well as temporary remission of disease symptoms without serious toxicity (3). The results obtained in this study and data from a phase I trial with TPA at the Cancer Institute of New Jersey in New Brunswick (4,5), indicated an acceptable toxicity profile, but beneficial effects in the leukemia patients were not observed in the New Brunswick study (4,5). Why the New Brunswick study differed from the study in China is not known but may be related to different dosing regimens in the two studies or to the low dose of cytosine arabinoside given together with TPA in the Chinese study. In additional studies, we found that clinically achievable concentrations of TPA in combination with all-trans retinoic acid (ATRA), 1α,25-dihydroxyvitamin D_3, sodium butyrate or capsaicin synergistically inhibited the growth and stimulated the differentiation of cultured HL-60 myeloid leukemia cells, suggesting that combinations of these drugs together with TPA may be more effective than TPA

alone for the treatment of refractory myeloid leukemia patients (6,7).

Previous studies showed that TPA inhibited the growth and induced apoptosis in androgen-dependent prostate cancer LNCaP cells (8-10). Studies from our laboratory showed that TPA alone or in combination with ATRA or with paclitaxel inhibited the growth of androgen-dependent LNCaP xenograft tumors in immunodeficient mice (11,12). Although androgen-dependent LNCaP prostate cancer cells are sensitive to TPA-induced growth inhibition and apoptosis, androgen-independent prostate cancer PC-3 cells are partially resistant to TPA (10,13). It is known that PC-3 cells have constitutive activation of NF-κB (14,15) and that NF-κB is an important regulator of growth and apoptosis in a variety of cells including prostate cancer cells (15-17). Activation of NF-κB has been shown to protect cells from apoptotic cell death (16,17), and blocking NF-κB activation enhances TPA-induced apoptosis in Jurkat leukemia T cells (18) and in myeloid leukemia cells (19). These results suggest that a combination of TPA and a pharmacological inhibitor of NF-κB may improve the therapeutic efficacy of TPA in prostate cancer cells.

We hypothesized that the partial resistance of PC-3 cells to TPA-induced growth inhibition and apoptosis is mediated by NF-κB activation and that inhibition of NF-κB will enhance the anticancer activity of TPA in these cells. To test this hypothesis, we investigated the effects of TPA alone or in combination with a pharmacological inhibitor of NF-κB, BAY, on growth and apoptosis in PC-3 cells cultured *in vitro* or grown as xenograft tumors in immunodeficient NCr mice. We found that BAY inhibited NF-κB activation and strongly enhanced TPA-induced growth inhibition and apoptosis in cultured PC-3 cells. We also found that a combination of TPA and BAY had a more potent inhibitory effect on the growth of PC-3 tumors in NCr immunodeficient mice than either agent alone.

Materials and methods

Cell culture and reagents. PC-3 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). TPA was obtained from Alexis Co. (San Diego, CA). (*E*)3-[(4-methylphenyl)-sulfonyl]-2-propenenitrile (BAY 11-7082; BAY) was obtained from Calbiochem (San Diego, CA). Propylene glycol, polysorbate 80, benzyl alcohol, ethanol, phosphate-buffered saline (PBS) and DMSO were purchased from Sigma (St. Louis, MO). Matrigel was obtained from BD Biosciences (Bedford, MA). RPMI-1640 tissue culture medium, penicillin-streptomycin, L-glutamine and fetal bovine serum (FBS) were from Gibco (Grand Island, NY). PC-3 cells were maintained in RPMI-1640 culture medium containing 10% FBS that was supplemented with penicillin (100 units/ml)-streptomycin (100 μg/ml) and L-glutamine (300 μg/ml). Cultured cells were grown at 37˚C in a humidified atmosphere of 5% CO_2 and were passed twice a week. PC-3 cells were initially seeded at a density of $0.2x10^5$ cells/ml in 35-mm tissue culture dishes (2 ml/dish) for assays of proliferation and apoptosis.

Determination of the number of viable cells. The number of viable cells after each treatment was determined using a hemacytometer under a light microscope (Nikon Optiphot, Japan). Cell viability was determined by the trypan blue exclusion assay, which was done by mixing 80 μl of cell suspension and 20 μl of 0.4% trypan blue solution for 2 min. Blue cells were counted as dead cells and the cells that did not absorb dye were counted as live cells.

Morphological assessment of apoptotic cells. Apoptosis was determined by morphological assessment in cells stained with propidium iodide (11). Briefly, cytospin slides were prepared after each experiment and cells were fixed with acetone/methanol (1:1) for 10 min at room temperature, followed by 10 min with propidium iodide staining (1 μg/ml in PBS) and analyzed using a fluorescence microscope (Nikon Eclipse TE200, Japan). Apoptotic cells were identified by classical morphological features including nuclear condensation, cell shrinkage, and formation of apoptotic bodies (11). At least 200 cells were counted in each sample and the percentage of apoptotic cells was determined.

NF-κB-dependent reporter gene expression assay. NF-κB transcriptional activity was measured by an NF-κB-luciferase reporter gene expression assay (20). An NF-κB luciferase construct was stably transfected into PC-3 cells and a single stable clone, PC-3 C4 (20), was used in the present study. In brief, PC-3 C4 cells were treated with TPA or BAY alone or in combination for 24 h, and the NF-κB-luciferase activities were measured using luciferase assay kits from Promega (Madison, WI). After treatment, the cells were washed with ice-cold PBS and harvested in a reporter lysis buffer. After centrifugation, 10 μl aliquots of the supernatants were used for measuring luciferase activity with a luminometer from Turner Designs Instruments (Sunnyvale, CA). The luciferase activity was normalized against protein concentration and expressed as percent of luciferase activity in the control cells, which were treated with DMSO solvent. The protein level was determined by Bio-Rad protein assay kits (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

PC-3 xenograft tumors in immunodeficient mice. Male NCr immunodeficient mice (6-7 weeks old) were obtained from Taconic farms Inc. (Germantown, NY). The animals were housed in sterile filter-capped microisolator cages and provided with sterilized food and water. Prostate cancer PC-3 cells ($2.0x10^6$ cells/0.1 ml/mouse) suspended in 50% Matrigel (Collaborative Research, Bedford, MA) in RPMI-1640 medium were injected subcutaneously into the right flank of the mice. After ~4 weeks, mice with well-established tumors (0.6-1.0 cm long and 0.6-1.0 cm wide) were injected i.p. with vehicle (5 μl/g body weight), TPA (100 ng/g; 5 μl vehicle/g), BAY (4 μg/g; 5 μl vehicle/g), TPA (100 ng/g) + BAY (4 μg/g) once a day for 36 days. Animals in the different experimental groups received the same amount of vehicle (5 μl/g body weight) which consisted of propylene glycol, polysorbate 80, benzyl alcohol, ethanol and water (40: 0.5: 1: 10: 48.5). Tumor size (length x width) and body weight were measured every third day. At the end of the study, mice were sacrificed, tumors were excised, weighed and placed in phosphate-buffered formalin at room temperature for 48 h and then placed in ethanol for 48 h before preparing paraffin sections as previously

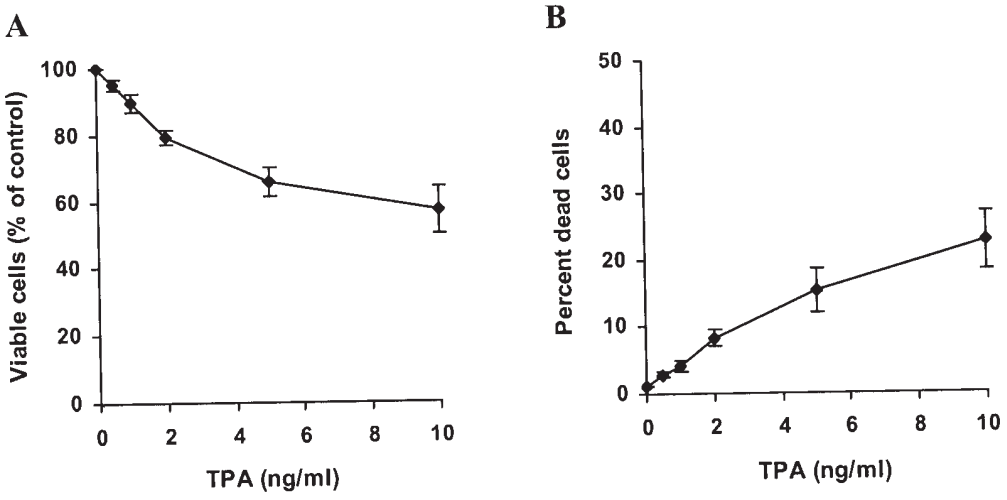

Figure 1. Effects of TPA on the growth and death of PC-3 cells. PC-3 cells were seeded at a density of $0.2x10^5$ cells/ml in 35-mm tissue culture dishes and incubated for 24 h. The cells were then treated with various concentrations of TPA (0.5-10 ng/ml) for 96 h. (A) The number of viable cells after treatment with TPA is expressed as percent of control. (B) The percent of dead cells was determined by a trypan blue exclusion assay. Each value is the mean ± SE from three separate experiments.

Table I. Effects of TPA alone or in combination with BAY on the growth and apoptosis of PC-3 cells

Treatment	No. of viable cells ($1x10^{-4}$)	Percent apoptotic cells
Control	45.1±1.2	1.1±0.2
TPA (0.5 ng/ml)	42.7±1.1	2.9±0.2
BAY (0.2 μg/ml)	44.2±0.7	1.6±0.4
BAY (0.4 μg/ml)	40.4±0.7	9.6±0.4
BAY (1.0 μg/ml)	35.2±1.0	18.3±1.4
TPA (0.5 ng/ml) + BAY (0.2 μg/ml)	38.7±1.0	8.2±0.6
TPA (0.5 ng/ml) + BAY (0.4 μg/ml)	34.7±0.9	17.5±1.9
TPA (0.5 ng/ml) + BAY (1.0 μg/ml)	20.4±1.7	30.1±1.8

PC-3 cells were seeded at a density of $0.2x10^5$ cells/ml in 35-mm tissue culture dishes and incubated for 24 h. The cells were then treated with TPA (0.5 ng/ml) alone or in combination with BAY (0.2, 0.4 and 1.0 μg/ml) for 96 h. The number of viable cells was determined by a trypan blue exclusion assay. Apoptotic cells were determined by morphological assessment. Each value represents the mean ± SE.

described (11). The animal experiment was carried out under an institutional animal care and use committee (IACUC)-approved protocol.

Statistical analyses. The analysis of variance (ANOVA) method with the Tukey-Kramer multiple comparison test (21) was used for the comparison of tumor size and body weight among different treatment groups at the end of the treatment.

Results

Effects of TPA alone or in combination with BAY on the growth and apoptosis of PC-3 cells. We first determined the effects of TPA on the growth and death of PC-3 cells. PC-3 cells were treated with different concentrations of TPA (0.5-10 ng/ml) for 96 h. The number of viable and dead cells was determined by a trypan blue exclusion assay (11). Treatment of PC-3 cells with TPA (0.5-10 ng/ml) for 96 h resulted in a decrease in the number of viable cells in a concentration-dependent manner (Fig. 1A). TPA (0.5-10 ng/ml) caused a 5-43% decrease in the number of viable PC-3 cells (Fig. 1A) and resulted in 3-23% dead cells (Fig. 1B). DMSO-treated control cells had only ~1% cell death.

The effects of TPA in combination with BAY on the growth and apoptosis of PC-3 cells were also studied. A trypan blue exclusion assay was used to determine the number of viable cells, and apoptosis was determined by using a morphological assessment of propidium iodide stained cells (11).

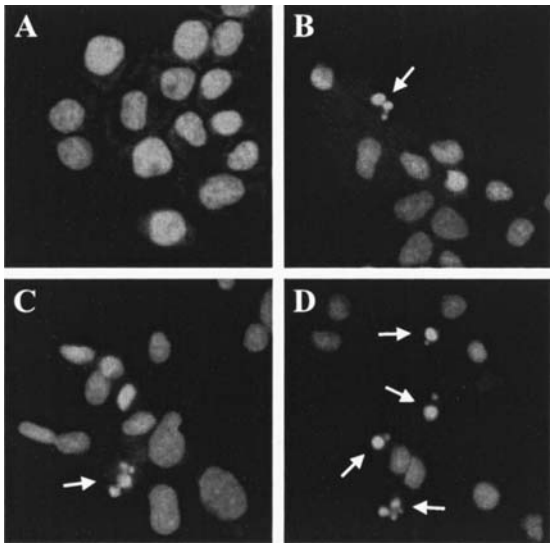

Figure 2. Effect of TPA alone or in combination with BAY on apoptosis in PC-3 cells. PC-3 cells were seeded at a density of $0.2x10^5$ cells/ml in 35-mm tissue culture dishes and incubated for 24 h. The cells were then treated once with TPA (0.5 ng/ml) alone or in combination with BAY (1 μg/ml) for 96 h. The cells were fixed with methanol/acetone (1:1) for 10 min and stained with propidium iodide (1 μg/ml) for 10 min. Apoptotic cells were determined by morphological assessment using a fluorescence microscope. (A) Control PC-3 cells. (B) PC-3 cells treated with TPA (0.5 ng/ml). (C) PC-3 cells treated with BAY (1 μg/ml). (D) PC-3 cells treated with a combination of TPA (0.5 ng/ml) and BAY (1 μg/ml).

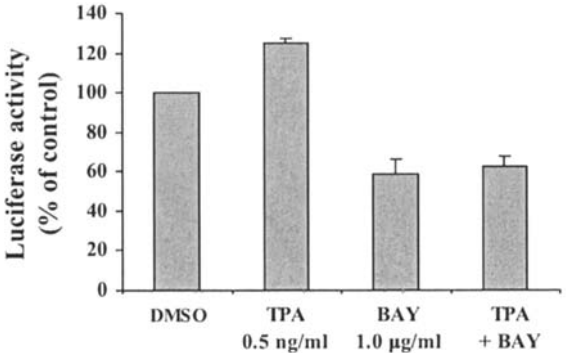

Figure 3. Effect of TPA or BAY alone or in combination on the activation of NF-κB in PC-3 cells. PC-3 C4 cells were seeded at a density of $0.2x10^6$ cells/ml of medium in 60-mm culture dishes (6 ml/dish) and incubated for 24 h. The cells were then treated with TPA (0.5 ng/ml) alone or in combination with BAY (1 μg/ml) for 24 h. The NF-κB transcriptional activity was measured by a luciferase activity assay. Each value is the mean ± SE from three separate experiments.

In these experiments, PC-3 cells were treated with TPA (0.5 ng/ml) alone or in combination with BAY (0.2, 0.4 and 1 μg/ml) for 96 h. Treatment with TPA (0.5 ng/ml) alone only had a small effect on the growth and apoptosis of PC-3 cells (Table I). BAY enhanced the inhibitory effect of TPA on growth and the stimulatory effect of TPA on apoptosis. The effect of TPA alone or in combination with BAY on morphologically distinct apoptotic cells from representative samples is shown in Fig. 2A-D. A combination of TPA (0.5 ng/ml) and BAY (1 μg/ml) caused a stronger induction of apoptosis in PC-3 cells than either agent alone (Fig. 2D).

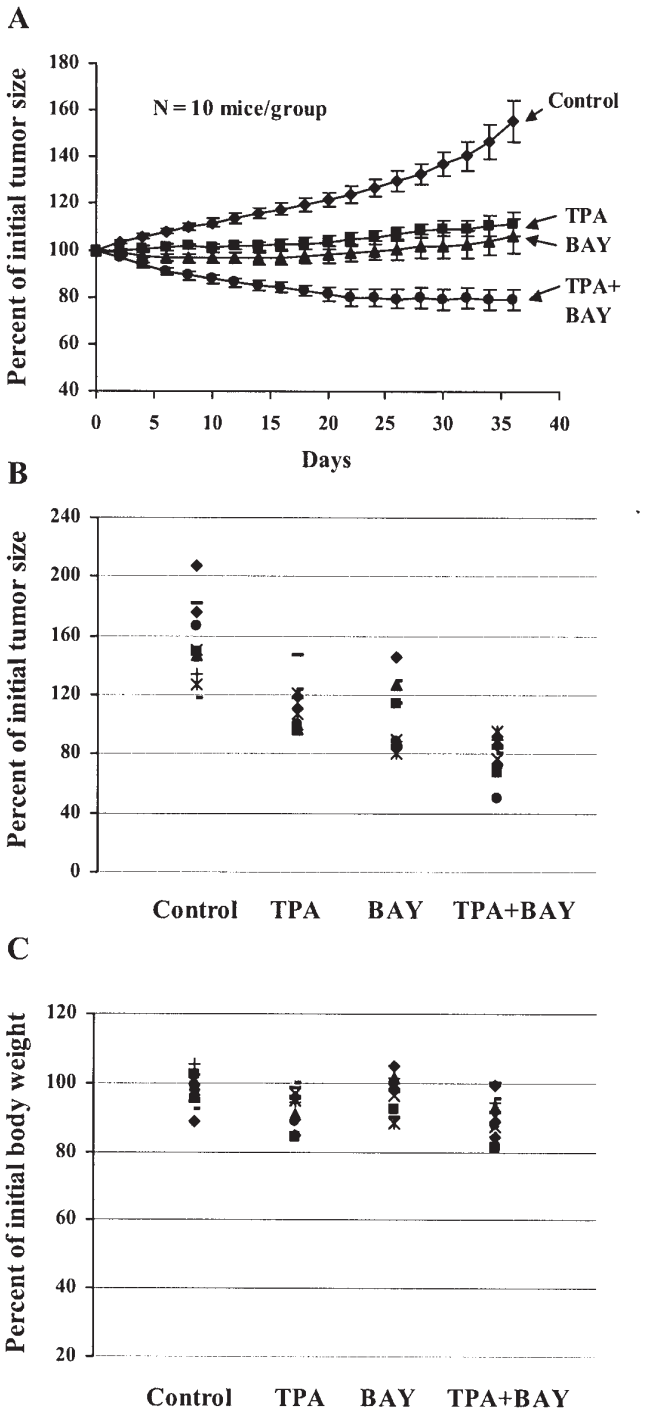

Figure 4. Effects of i.p. injections of TPA alone or in combination with BAY on the growth of PC-3 tumors and the body weight of NCr immunodeficient mice. Male NCr immunodeficient mice with well-established tumors (0.65-1.0 cm long and 0.65-1.0 cm wide) were injected i.p. with vehicle (5 μl/g body weight), TPA (100 ng/g; 5 μl vehicle/g), BAY (4 μg/g; 5 μl vehicle/g) or TPA (100 ng/g) + BAY (4 μg/g) once a day for 36 days. Tumor size (length x width) was measured and expressed as percent of initial tumor size. (A) Growth curve of PC-3 tumors in each group. Each value represents the mean ± SE. (B) Individual tumor size after treatment for 36 days. (C) Individual body weight of mice after treatment for 36 days.

Effect of BAY on NF-κB activity in PC-3 cells. We used an NF-κB-luciferase reporter gene expression assay in PC-3 C4 cells (20) to determine the effect of BAY on the activation of

Table II. Effects of i.p. injections of TPA alone or in combination with BAY on the growth or regression of PC-3 prostate tumors in NCr mice.

Treatment	Number of mice	Percent of animals with tumor growth	Percent of animals with tumor regression
Vehicle control	10	100	0
TPA	10	60	40
BAY	10	50	50
TPA + BAY	10	0	100

Male NCr immunodeficient mice with PC-3 tumors were injected i.p. once a day with vehicle, TPA (100 ng/g), BAY (4 μg/g) or TPA (100 ng/g) + BAY (4 μg/g) in vehicle as described in Materials and methods. The effects of the various treatments on tumor growth or regression in individual mice are indicated.

Table III. Effects of i.p. injections of TPA alone or in combination with BAY on the percent of mitotic cells and caspase 3 (active form) positive cells in PC-3 tumors.

Treatment	Number of animals	Percent mitotic cells	Percent caspase 3-positive cells	Ratio of percent mitotic cells/ caspase 3-positive cells
Control	10	0.56±0.03	0.32±0.02	1.84±0.16
TPA	10	0.46±0.03[a]	0.43±0.02[a]	1.06±0.07[b]
BAY	10	0.44±0.02[a]	0.42±0.02[a]	1.08±0.09[b]
TPA + BAY	10	0.35±0.02[b]	0.47±0.03[b]	0.81±0.10[b]

Male NCr immunodeficient mice with PC-3 tumors were injected i.p. once a day with vehicle, TPA (100 ng/g), BAY (4 μg/g) or TPA (100 ng/g) + BAY (4 μg/g) in vehicle as described in Materials and methods. The animals were sacrificed at 24 h after the last injection. Mitotic cells were determined with a light microscope in hematoxylin/eosin stained tissue sections. Caspase 3 (active form) positive cells were determined immunohistochemically as described in Materials and methods. Each value represents the mean ± SE. Statistical analysis was done by using ANOVA with the Tukey-Kramer multiple comparison test. Statistical differences from the control are indicated. [a]$p<0.05$, [b]$p<0.001$.

NF-κB. PC-3 C4 is a cell line derived from the stable transfection of PC-3 cells with an NF-κB luciferase construct (20). In these experiments, PC-3 C4 cells were treated with BAY (1 μg/ml) in the presence or absence of TPA for 24 h. Treatment of PC-3 C4 cells with TPA (0.5 ng/ml) resulted in a small increase in NF-κB activity (Fig. 3). BAY (1 μg/ml) alone caused a strong decrease in NF-κB activity. The stimulatory effect of TPA on NF-κB activity was strongly suppressed by BAY (Fig. 3).

Effects of i.p. injections of TPA alone or in combination with BAY on the growth of PC-3 tumors in NCr nude mice. To further investigate the effect of TPA alone or in combination with BAY on the growth of PC-3 tumors, NCr nude mice with well established PC-3 tumors (~0.6-1.0 cm long and 0.6-1.0 cm wide) were injected i.p. with vehicle (5 μl/g body weight), TPA (100 ng/g; 5 μl vehicle/g), BAY (4 μg/g; 5 μl vehicle/g), TPA (100 ng/g) + BAY (4 μg/g) once a day for 36 days. The effects of i.p. injections of TPA alone or in combination with BAY on tumor growth and body weight are described in Fig. 4. At the end of the experiment, the mean ± SE for percentage of initial tumor size was 155.7±8.7 for the control group, 111.6±5.1 for the TPA group, 106.4±7.1 for the BAY group and 79.1±4.2 for the TPA + BAY group (Fig. 4B). Statistical analysis using ANOVA with the Tukey-Kramer multiple comparison test showed that the differences in the percent of initial tumor size between the control group and the TPA group, between the control group and the BAY group or between the control group and the TPA + BAY group were statistically significant ($p<0.001$). The average tumor size in the TPA + BAY group was significantly smaller than that in the TPA ($p<0.01$) or BAY group ($p<0.05$).

The percent of animals with continued tumor growth in tumor-bearing mice treated with vehicle, TPA, BAY or BAY + TPA was 100, 60, 50 and 0%, respectively (Table II). The results indicated that all ten animals treated with TPA + BAY had some tumor regression whereas none of the control animals treated with vehicle had tumor regression. The effect of the various treatments on body weight is shown in Fig. 4C. The mean ± SE for the percentage of initial body weight was 97.6±1.5 for the vehicle treated control group, 92.9±1.8 for the TPA group, 98.2±1.4 for the BAY group and 91.2±2.0 for

the TPA + BAY group. Statistical analysis using ANOVA with the Tukey-Kramer multiple comparison test showed that the differences in the percent of initial body weight between the control group and any of the treatment group were not statistically significant ($p>0.05$).

Effects of i.p. injections of TPA alone or in combination with BAY on proliferation and apoptosis in PC-3 tumors in NCr nude mice. The effects of daily i.p. injections of TPA alone or in combination with BAY for 36 days on proliferation and apoptosis in PC-3 tumors described in Fig. 4 were studied by determining mitotic cells and caspase 3 (active form) positive cells in these tumors. The percentage of mitotic cells was decreased significantly in tumors from mice treated with TPA ($p<0.05$), BAY ($p<0.05$) or TPA + BAY ($p<0.001$) when compared to the control group (Table III). Apoptosis as determined by caspase 3 (active form) positive cells in tumors was increased significantly in tumors from mice treated with TPA ($p<0.05$), BAY ($p<0.05$) or TPA + BAY ($p<0.001$) when compared to the control group (Table III). The ratio of the percent mitotic cells/percent caspase 3 (active form) positive cells which is an index of the balance between cell proliferation and cell death in the PC-3 tumors was also calculated. As shown in Table III, the ratio of the percent mitotic cells/percent caspase 3 (active form) positive cells was decreased significantly in all treatment groups as compared to the control group ($p<0.001$).

Discussion

In the present study, we demonstrated that TPA modestly inhibited the growth and induced apoptosis in androgen-independent prostate cancer PC-3 cells cultured *in vitro* and grown as xenograft tumors in immunodeficient mice. We also demonstrated that BAY, an NF-κB inhibitor, significantly increased TPA-induced growth inhibition and apoptosis in PC-3 cells. Early stage prostate cancer requires androgen for growth and thus responds to androgen deprivation therapy. However, the disease progresses to an androgen-independent state that is unresponsive to androgen ablation (22,23). Treatment of these hormone-refractory prostate cancer patients with chemotherapeutic agents is generally unsatisfactory because the development of resistance to apoptosis in advanced prostate cancer cells makes chemotherapeutic agents ineffective in treating the disease (23-25). Therefore, it is an important challenge to develop effective ways for the treatment of androgen-independent prostate cancer. Studies from our laboratory and others showed that TPA induced growth inhibition and apoptosis in androgen-dependent LNCaP prostate cancer cells (8,9,11). TPA in combination with radiation was shown to induce apoptosis in LNCaP xenograft tumors (26). More recently, we found that TPA alone or in combination with ATRA or with paclitaxel suppressed the growth of LNCaP tumors in immunodeficient mice (11,12). In the present study, we demonstrated that TPA suppressed the growth of androgen-independent PC-3 prostate tumors in immunodeficient mice, and a combination of TPA and BAY caused PC-3 tumor regression without toxicity as reflected by the lack of a significant body weight decrease (Fig. 4C). Since our earlier studies in myeloid leukemia patients indicated an acceptable toxicity profile for TPA (3-5), clinical studies using TPA alone or in combination with pharmacological inhibitors of NF-κB in prostate cancer patients may be warranted.

NF-κB is an important cellular regulator of growth and apoptosis. This transcription factor has been associated with multiple aspects of oncogenesis, including the control of apoptosis, cell cycle, differentiation, and cell migration (15,17). Numerous studies have indicated that NF-κB activation can suppress cell death pathways and that NF-κB activation is required to protect cells from apoptotic cascade induced by TNFα and other stimuli (16,17). Earlier studies have shown that activated NF-κB is absent in normal prostate and prostatic intraepithelial neoplasia lesions (PIN), whereas it is commonly present in invasive prostate cancer (27-29). Constitutive activation of NF-κB is related to prostate cancer progression (29). Moreover, NF-κB activation predicts a high risk of relapse in patients with localized disease (28,30). Therefore, NF-κB may serve as a therapeutic target for the development of new anticancer agents. In earlier studies, inhibition of NF-κB by BAY or a related inhibitor in cultured cancer cells or *in vivo* in mice inhibited tumor growth and enhanced apoptosis (31-35). In the present study, we used BAY which inhibits IκBα phosphorylation and thus decreases NF-κB activation (36). Our results showed that BAY inhibited NF-κB activation in PC-3 cells (Fig. 3), and treatment of cultured PC-3 cells with BAY resulted in growth inhibition and apoptosis (Table I and Fig. 2). Daily i.p. injection of BAY in immunodeficient mice inhibited the growth of PC-3 tumors (Fig. 4). Administration of a combination of TPA and BAY caused PC-3 tumor regression in these mice. These results suggest that targeting NF-κB is an effective strategy for treating prostate cancer or improving the anticancer activity of TPA in prostate cancer.

Many cancer chemotherapeutic agents such as docetaxel (37), irinotecan (38) and 5-fluorouracil (39) were reported to activate NF-κB in cancer cells. Activation of NF-κB may be a protective response of cancer cells to chemotherapeutic agents that leads to the development of resistance to chemotherapy, and inhibition of NF-κB was shown to enhance the anticancer activity of certain chemotherapeutic agents (37-39). TPA was shown to induce the activation of NF-κB (40,41), and our recent study showed that inhibition of NF-κB was associated with enhanced TPA-induced apoptosis in myeloid leukemia cells (19). In the present study, we found that treatment of the PC-3 C4 cells with a low concentration of TPA (0.5 ng/ml) resulted in an increase in NF-κB transcriptional activity (Fig. 3). Inhibition of NF-κB activity by BAY strongly increased TPA-induced growth inhibition and apoptosis in cultured PC-3 cells. Administration of a combination of TPA and BAY also had a more potent inhibitory effect on the growth of PC-3 tumors in NCr immunodeficient mice than TPA alone (Fig. 4). Inhibition of NF-κB by suitable pharmacological inhibitors may be an effective strategy for improving the therapeutic efficacy of TPA in prostate cancer.

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