[CANCER RESEARCH 63, 107–110, January 1, 2003]

Suppression of Hormone-refractory Prostate Cancer by a Novel Nuclear Factor κB Inhibitor in Nude Mice

Eiji Kikuchi, Yutaka Horiguchi,[1] Jun Nakashima, Kenji Kuroda, Mototsugu Oya, Takashi Ohigashi, Nozomu Takahashi, Yutaka Shima, Kazuo Umezawa, and Masaru Murai

Department of Urology, Keio University, School of Medicine, Tokyo 160-8582 [E. K., Y. H., J. N., K. K., M. O., T. O., M. M.], and Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Yokohama 223-0061 [N. T., Y. S., K. U.] Japan

ABSTRACT

We have synthesized and explored the feasibility of using a novel nuclear factor (NF) κB inhibitor, a dehydroxymethylepoxyquinomicin designated as DHMEQ, against prostate cancer. The activity of NFκB, evaluated by transient transfection of a luciferase reporter DNA containing a specific binding sequence for NFκB, was inhibited by DHMEQ in three human hormone-refractory prostate cancer cell lines, DU145, JCA-1, and PC-3. Statistically significant growth inhibition was achieved by 20 μg/ml of DHMEQ, and marked levels of apoptosis were induced 48 h after DHMEQ administration *in vitro*. Electrophoretic mobility shift assay showed that DHMEQ completely inhibited NFκB DNA binding activity in JCA-1 cells. Furthermore, i.p. administrations of DHMEQ significantly inhibited pre-established JCA-1 s.c. tumor growth in nude mice without any side effects. Our result indicates the possibility of using a novel NFκB activation inhibitor, DHMEQ, as a new treatment strategy against hormone-refractory prostate cancer.

INTRODUCTION

Hormone-refractory prostate cancer cell lines have been known to produce multiple cytokines including IL-1α,[2] IL-6, and granulocyte colony-stimulating factor, which are thought to play a crucial role in tumor growth and progression (1). Previously, it has been demonstrated that both JCA-1 and PC-3 hormone-refractory prostate cancer cells secreted large amounts of IL-6, which was enhanced by exogenous TNF-α (2). Moreover, we have reported recently that the serum level of IL-6 was a significant prognostic factor for prostate cancer patients, especially with disseminated diseases (3). These cytokines that might enhance the malignant potential of hormone-refractory prostate cancer are regulated by several transcription factors through various signal transduction pathways. Among such transcription factors, NFκB is one of the most critical regulators of cytokine-inducible gene expression (4). It is well known that NFκB is often constitutively activated in hormone-refractory prostate cancer cells, which may increase expression of antiapoptosis proteins, thereby decreasing the effectiveness of anticancer therapy and contributing to the development of the chemoresistant phenotypes (5, 6). Palayoor *et al.* (5) have reported that NFκB is constitutively activated in the hormone-refractory prostate cancer cell lines PC-3 and DU145, but not in the hormone-dependent LNCaP prostate cancer cell line. It is suggested that TNF-α did not induce cytotoxic effects on hormone-refractory prostate cancer cells, because the NFκB protective pathway prevents the cells from undergoing apoptosis (7). These findings support the hypothesis that NFκB plays an important role in maintaining the chemoresistant nature of hormone-refractory prostate cancers. Therefore, approaches that inhibit NFκB function may be beneficial in the treatment of chemoresistant prostate cancers. On the basis of this understanding, we have investigated the effectiveness of a newly synthesized unique NFκB inhibitor as a potent treatment modality for hormone-refractory prostate cancers.

Epoxyquinomicin C was originally isolated from *Amycolatopsis* as a weak antibiotic and anti-inflammatory agent, having a 4-hydroxy-5, 6-epoxycyclohexenone structure like panepoxydone (8). Panepoxydone isolated from the basidiomycete *Lentinus crinitus* was found to inhibit TNF-α-induced activation of NFκB (9). Therefore, we have newly designed and synthesized DHMEQ as a 5-dehydroxymethyl derivative of epoxyquinomicin C, which showed anti-NFκB activity in cultured human leukemia Jurkat cells and inhibited type II collagen-induced rheumatoid arthritis in mice (10). Recently, DHMEQ was found to inhibit activation of NFκB at the level of nuclear translocation (11). Thus far, in the field of anticancer research, no investigations have been reported to regulate NFκB activation using a single agent synthesized from a natural product. In the present study, we evaluated the inhibitory effects of a novel NFκB activation inhibitor, DHMEQ, against hormone-refractory prostate cancer cells on: (*a*) constitutive activation of NFκB; (*b*) the levels of antiapoptosis; and (*c*) tumor growth in a xenograft animal model to establish an innovative and effective treatment strategy for hormone-refractory prostate cancers.

MATERIALS AND METHODS

Cell Lines. Androgen-dependent prostate cancer cell line, LNCaP (American Type Culture Collection, Manassas, VA), as well as androgen-insensitive prostate cancer cell lines, DU145, PC-3 (American Type Culture Collection), and JCA-1 (12), were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 μg/ml streptomycin (Life Technologies, Inc., Grand Island, NY), and 100 IU/ml penicillin (Life Technologies, Inc.).

Chemicals. DHMEQ was synthesized in our laboratory (10). It was dissolved in DMSO to prepare a 10 μg/ml solution and subsequently diluted in culture medium to a final DMSO concentration of <0.1%.

Cell Growth Assay. The growth-inhibitory effects of DHMEQ were determined as reported previously (13). Briefly, 2×10^4 cells were seeded in each well of 96-well plates and allowed to grow overnight. Then, cells were treated with various concentrations of DHMEQ. Cells treated with the same concentrations of DMSO were served as controls. After 48 h of incubation, cytotoxicity was determined by staining the plates with 0.2% crystal violet. The absorbance value of each well was determined at 550 nm with a 405 nm reference beam by a microplate reader (Bio-Rad, Tokyo, Japan).

Measurement of Activity of NFκB. The activity of NFκB was monitored using a luciferase plasmid DNA that contains a specific binding sequence for NFκB (10). Transfection was carried out using cationic liposome, Gene PORTER 2 (Gene Therapy Systems, San Diego, CA). Briefly, 2×10^4 cells per each well were plated in 96-well plates. When cultured cells reached ~80% confluence, cells were overlaid with 0.2 μg DNA/1 μl Gene PORTER 2 complexes in a total volume of 50 μl of serum-free RPMI 1640 for 14 h. Subsequently, various concentrations of DHMEQ in 50 μl of RPMI 1640 containing 10% fetal bovine serum were added. After additional incubation for 8 h, cells were washed with PBS and harvested in 50 μl of Reporter Lysis Buffer provided in Luciferase Assay kit (Promega, Madison, WI). Luciferase activity in 10 μl of lysate was measured by a luminometer (Lumat 9501;

Received 2/20/02; accepted 10/29/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at Department of Urology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Phone: 81-3-5363-3825; Fax: 81-3-3225-1985; E-mail: horigchi@aol.com.

[2] The abbreviations used are: IL, interleukin; TNF, tumor necrosis factor; NFκB, nuclear factor κB; DHMEQ, dehydroxymethylepoxyquinomicin; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; PI, propidium iodide; EMSA, electrophoretic mobility shift assay.

Berthold). Obtained raw luciferase activities were normalized by dividing with percent cytotoxicity at each concentration.

EMSA. JCA-1 cells treated with 20 μg/ml of DHMEQ were incubated for different time periods. The cells were harvested, washed with PBS, suspended in 400 μl of buffer A (10 mM HEPES, 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, and 0.1 mM phenylmethylsulfonyl fluoride), and incubated on ice for 15 min. Nuclei were pelleted by centrifugation for 5 min at 14,000 rpm, resuspended in 40 μl of buffer C [50 mM HEPES, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, and 25% glycerol (v/v)], incubated on ice for 20 min, and centrifuged for 5 min at 14,000 rpm at 4°C. The supernatant was used as a nuclear extract. The binding reaction mixture containing 5 μg of protein of nuclear extract, 2 μg of poly(deoxyinosinic-deoxycytidylic acid), and ^{32}P-labeled probe was incubated for 20 min at room temperature. The complexes were separated from free DNA on 4% native polyacrylamide gel. The DNA probe used for NFκB binding was the double-stranded oligonucleotide containing the κB site from the mouse κ light chain enhancer (5′-ATGTGAGGGGACTTTCCCAGGC-3′).

Western Blot Analysis. The expression levels of IκBα, Bcl-2, and Bcl-xL protein in JCA-1 cells were determined by Western blot analysis. Samples containing equal amounts of protein (30 μg) were subjected to electrophoresis on a SDS polyacrylamide gel and transferred to a nitrocellulose filter. The filters were blocked with Tris-buffered saline containing 5% nonfat milk at 4°C overnight and then incubated for 1 h with anti-IκBα rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Bcl-2 mouse monoclonal antibody, anti-Bcl-xL mouse monoclonal antibody (Transduction Laboratories, Lexington, KY), or anti-β-actin mouse antibody (Sigma Chemical Co., St. Louis, MO). The filters were then incubated for 1 h with antirabbit or antimouse secondary antibody (Dako A/S, Glostrup, Denmark), and reactivity was detected by enhanced chemiluminescence system (Amersham Life Science, Inc., Little Chalfont, United Kingdom).

Detection of Apoptosis. Quantification of apoptosis was determined using flow cytometric analysis of cells labeled with TUNEL assay. Cells (1×10^5) were plated in T-25 flask, incubated overnight, and treated with 20 μg/ml of DHMEQ for 48 h. TUNEL assay was performed using ApopTag kit (Intergen Co., Purchase, NY), and apoptosis was detected by flow cytometer (Beckman Coulter, Inc., Fullerton, CA). The staining by annexin V/PI, using Mebcyto Apoptosis kit (Medical & Biological Laboratories, Nagoya, Japan), was also used to monitor apoptosis in JCA-1 cells. Forty-eight h after the treatment with various concentrations of DHMEQ, cells were harvested, washed, and then incubated with annexin V-FITC and PI at room temperature for 15 min in the dark. The fluorescence was analyzed by flow cytometer, and subsequent analysis was carried out according to the manufacturer's instructions.

Treatments *in Vivo*. All of the procedures involving animals and their care in this study were approved by the animal care committee of Keio University in accordance with institutional and Japanese government guidelines for animal experiments. Male BALB/c-*nu/nu* mice were obtained from Sankyo Lab Service Co. (Tokyo, Japan). JCA-1 cells (5×10^6) were implanted s.c. in the flank of each nude mouse. When animals developed palpable tumors, mice were randomly assigned into two groups. Then 8 μg/kg DHMEQ was administered i.p. once daily for consecutive 14 days. Control animals only received vehicle medium injection. Each experimental group consisted of 8 mice. Animals were carefully monitored, and tumor size as well as body weight was measured weekly for 5 weeks. Tumor volume was calculated according to the formula $a^2 \times b \times 0.52$, where a and b are the smallest and largest diameters, respectively.

Statistical Analysis. The statistical analysis was performed using Student's *t* test. Unless otherwise indicated, average values were expressed as mean values with SD. *P*s < 0.01 were interpreted as statistically significant.

RESULTS AND DISCUSSION

Significant cytotoxic effects of DHMEQ were observed in DU145, JCA-1, and PC-3 cells treated with DHMEQ at concentrations of 10 μg/ml or higher, 2.5 μg/ml or higher, and 10 μg/ml or higher for 48 h, respectively ($P < 0.01$; Fig. 1*A*). DHMEQ at a concentration of 20 μg/ml inhibited cell growth of all of the prostate cancer cells in a time-dependent manner (Fig. 1*B*). DMSO, which was used as a solvent for DHMEQ, was not toxic even at the highest concentration

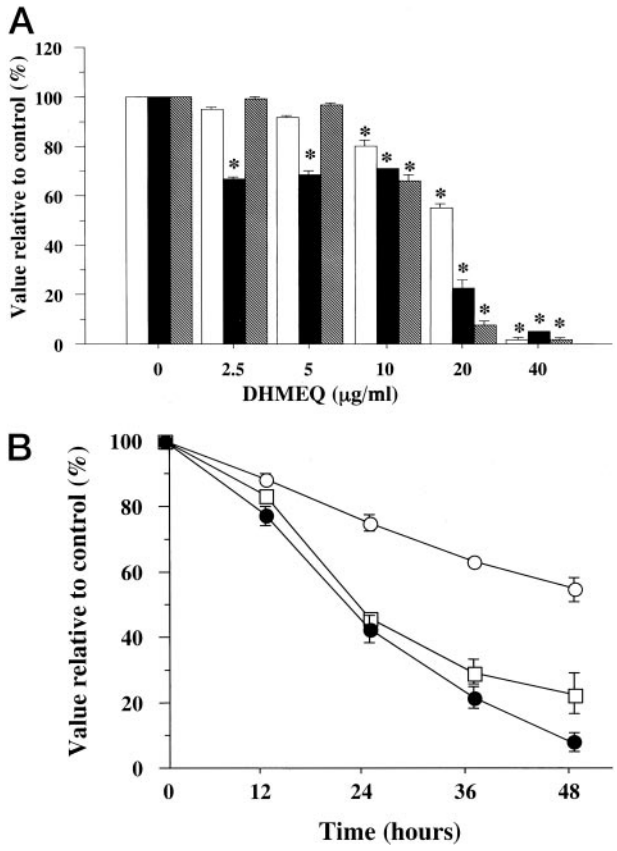

Fig. 1. Cytotoxic effects of DHMEQ on hormone-refractory prostate cancer cell lines. The cells [DU145 (□), JCA-1 (■), and PC-3 (▨)] were treated with various concentrations of DHMEQ (*A*). Cytotoxic effects of DHMEQ on various time points (*B*). The cells [DU145 (○), JCA-1 (□), and PC-3 (●)] were treated with 20 μg/ml of DHMEQ and incubated for various time periods. Control cells were treated with the same concentration of DMSO as used in DHMEQ treatment. The cell viabilities were measured by quantitative crystal violet assay. Each value represents the mean derived from at least three individual experiments; *bars*, ±SD. *, more statistically significant than controls ($P < 0.01$).

used in this study. In hormone-dependent LNCaP prostate cancer cells, DHMEQ did not induce significant growth inhibition at concentrations of 20 μg/ml or less (data not shown), suggesting that DHMEQ is more effective in hormone-refractory prostate cancer cells in which NFκB is constitutively activated. We performed a transient transfection assay using a NFκB luciferase reporter gene to evaluate the effect of DHMEQ on NFκB activity (Fig. 2, *A–C*). The significant decrease of NFκB activity was observed in DU145, JCA-1, and PC-3 cells treated with DHMEQ at concentrations of 10 μg/ml or higher, 2.5 μg/ml or higher, and 10 μg/ml or higher, respectively ($P < 0.01$). These results confirmed that DHMEQ inhibited constitutively activated NFκB in these hormone-refractory prostate cancer cells.

Analysis of NFκB binding activity to the κB DNA site in JCA-1 cells was examined by EMSA (Fig. 3). The DNA binding activity was completely inhibited after 2, 4, and 6 h of exposure to 20 μg/ml of DHMEQ. Then, 8 h after the DHMEQ treatment, DNA binding activity had recovered slightly. This result suggested that DHMEQ completely inhibited NFκB DNA binding activity in JCA-1 cells, in which NFκB was constitutively activated. Because the activation of NFκB normally proceeds through the ubiquitination and degradation of the IκBα inhibitory protein, Western blotting was carried out to evaluate the effects of DHMEQ on the expression levels of the IκBα protein in JCA-1 cells. IκBα was not modulated even by the highest dose of DHMEQ (Fig. 4). This result indicated that inhibition of NFκB activity in JCA-1 cells by DHMEQ was not associated with

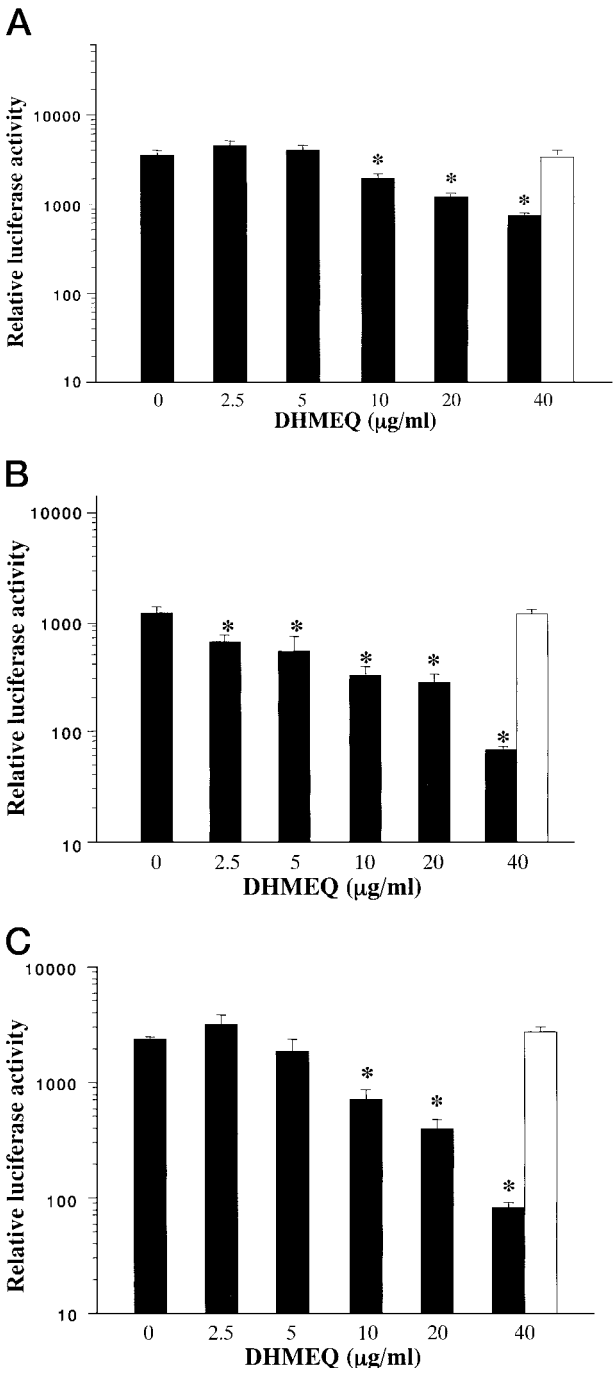

Fig. 2. NFκB luciferase assay for evaluating the effects of DHMEQ on NF-κB activity. Cells [DU145 (*A*), JCA-1 (*B*), and PC-3 (*C*)] were transfected with luciferase DNA and various concentrations of DHMEQ (■) were added. Cells treated with the highest concentration of DMSO were used as control (□). Luciferase assay was performed after 8 h of incubation with DHMEQ. Each value represents the mean derived from at least three individual experiments; *bars*, ±SD. *, more statistically significant than controls ($P < 0.01$).

phosphorylation and degradation of IκBα. The molecular target of DHMEQ on NFκB activity is still under investigation, however, we have already shown that DHMEQ inhibited TNF-α-induced activation of NFκB in human T-cell leukemia Jurkat cells possibly by inhibiting the nuclear translocation of NFκB from the cytoplasm (11).

There are several evidences that NFκB plays an important role in antiapoptotic mechanism. Previous studies have shown that the absence or inhibition of NFκB subunits in culture cells such as fibroblasts and macrophages resulted in potentiation of apoptosis in response to TNF-α (14, 15). In our current study, significant apoptosis was induced by DHMEQ (20 μg/ml) treatment in all of the three prostate cancer cell lines (Fig. 5). With staining by annexin V/PI, in JCA-1 cells, apoptotic indexes induced by 20 or 40 μg/ml of DHMEQ and DMSO were 44.1%, 23.8%, and 2.9%, respectively. Taken together, DHMEQ has the potent inhibitory effects on NFκB activation and produces a significant decrease in cell viabilities through apo-

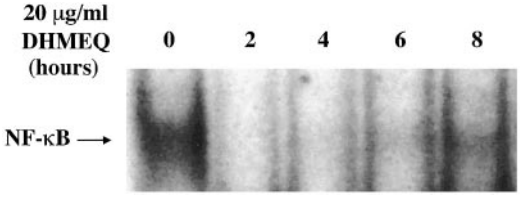

Fig. 3. Inhibition of NFκB DNA binding activity by DHMEQ treatment. JCA-1 cells were treated with 20 μg/ml of DHMEQ and incubated for various time periods. Then the nuclear extract was assayed by EMSA.

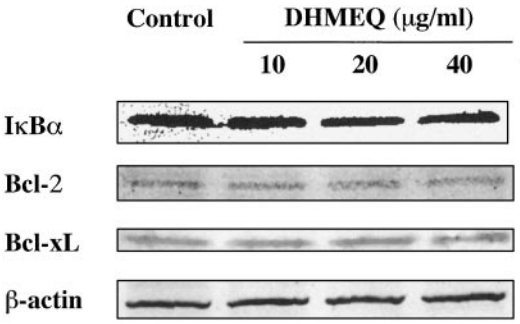

Fig. 4. The effects of DHMEQ on the protein expression levels of IκBα, Bcl-2, and Bcl-xL in JCA-1 cells. Western blotting was performed using antibodies specific for Bcl-2, Bcl-xL, IκBα, and β-actin.

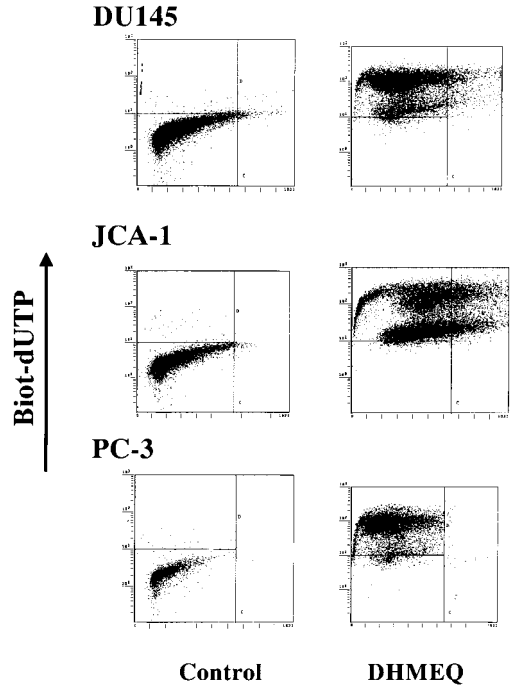

Fig. 5. Detection of apoptosis using flow cytometric analysis. Prostate cancer cells were treated with DHMEQ at concentration of 20 μg/ml for 48 h. TUNEL assay was performed, and apoptosis was detected by flow cytometry. Cells treated with DMSO were served as controls. *Upper left quadrants* of each graph represent the apoptotic cell populations.

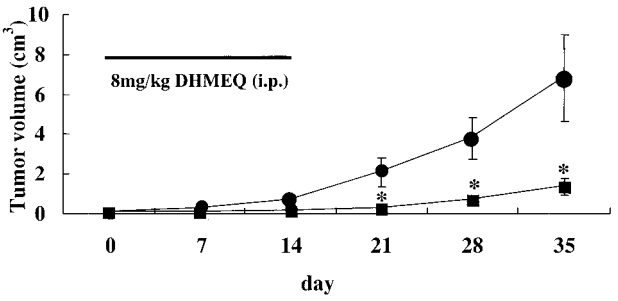

Fig. 6. Treatments *in vivo*. JCA-1 cells (5×10^6) were implanted s.c. in the flank of nude mice. When animals had developed palpable tumors, DHMEQ was administered i.p. once daily for 14 days. Control animals received vehicle medium administration. The mean tumor volumes in cm^3 are shown (●, control; ■, DHMEQ). *, more statistically significant than controls ($P < 0.01$); *bars*, ±SE.

ptotic mechanism. We also investigated whether Bcl-2 and Bcl-xL, which were important regulators of apoptosis, could be affected by DHMEQ treatment (Fig. 4). DHMEQ did not lower the levels of both Bcl-2 and Bcl-xL proteins, which suggested that DHMEQ might induce apoptosis through a different pathway from the Bcl-2 family.

Because of these encouraging findings *in vitro*, we tested cytotoxic effects of DHMEQ on JCA-1 s.c. tumors inoculated in nude mice. Significant decreases in the mean tumor volume were demonstrated in mice treated with DHMEQ (0.27 ± 0.12 cm^3) when compared with controls (2.07 ± 0.75 cm^3) 21 days after the start of the treatment ($P < 0.01$; Fig. 6). DHMEQ treatment at the dosage used was well tolerated, leading to no body weight loss in animals when compared with controls (data not shown).

Other approaches to inhibit NFκB activation including IκBα gene delivery have been investigated (16). A recombinant adenovirus vector expressing the stable form of IκBα clearly induced apoptosis, when cancer cells showed a constitutive NFκB activity *in vitro* (17). However, the clinical feasibility of gene therapy approaches for inhibiting NFκB are quite limited by requisite intratumoral delivery of a vector that expresses the NFκB inhibitor, and few studies reported the usefulness of this strategy in an *in vivo* model.

Nonsteroidal anti-inflammatory drugs could also inhibit the activation of NFκB. Dexamethasone activated intracellular IκB synthesis (18). Ibuprofen inhibited an upstream regulator of IκB kinase (19). Although these agents have been shown to specifically inhibit NFκB and have antitumor effect *in vitro*, again, *in vivo* study was not fully investigated. In reality, these agents may not be used as a single agent, rather they can be used in combination with other cytotoxic agents for clinical application.

Natural products reported to inhibit activation of NFκB are panepoxydone (9), cycloepoxydon (20), and gliotoxin (21). Panepoxydone and cycloepoxydon from the deuteromycete strain were suggested to inhibit TNF-α-induced phosphorylation and degradation of IκBα, and activation of NFκB. Gliotoxin produced by fungi was also reported to inhibit NFκB by preventing the degradation of IκBα. Few investigations were reported thus far in the literature to use such natural products that had inhibitory effects on NFκB activation in the field of cancer research as we clearly demonstrated in this study using DHMEQ.

Recently, it has been reported that there is some possibility that JCA-1 cells are cross-contaminant (22). However, it has also been suggested that a subline may have acquired certain phenotypic characteristics. At least NFκB is constitutively activated as clearly shown in our EMSA results in which untreated JCA-1 cells displayed elevated levels of NFκB DNA binding activity, as shown previously in PC-3 and DU145 cells (5). Furthermore, DHMEQ apparently inhibited the NFκB activities *in vitro* and the tumor growth *in vivo*.

In conclusion, our results indicate that DHMEQ inhibits NFκB activity resulting in promoting apoptotic mechanisms in hormone-refractory prostate cancer cell lines *in vitro* and has a potent inhibitory effect on JCA-1 tumor growth in animal models. This is the first report to certify the evidence that a single agent synthesized from a natural product has significant cytotoxic effects against hormone-refractory prostate cancers. These results demonstrated that a novel and unique NFκB inhibitor, DHMEQ, is a promising candidate as an antitumor agent in hormone-refractory prostate cancer. Additional studies on safety and efficacy of DHMEQ would provide a rationale for eventual clinical trails in hormone-refractory prostate cancer patients.

ACKNOWLEDGMENTS

We thank Azusa Yamanouchi and Hiroshi Nakazawa for excellent technical assistance in cell culture and flow cytometric analysis.

REFERENCES

1. Chung, T. D., Yu, J. J., Spiotto, M. T., Bartkowski, M., and Simons, J. W. Characterization of the role of IL-6 in the progression of prostate cancer. Prostate, *38:* 199–207, 1999.
2. Nakajima, Y., DelliPizzi, A. M., Mallouh, C., and Ferreri, N. R. TNF-mediated cytotoxicity and resistance in human prostate cancer cell lines. Prostate, *29:* 296–302, 1996.
3. Nakashima, J., Tachibana, M., Horiguchi, Y., Oya, M., Ohigashi, T., Asakura, H., and Murai, M. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin. Cancer Res., *6:* 2702–2706, 2000.
4. Baeuerle, P. A., and Baltimore, D. NF-κB: ten years after. Cell, *87:* 13–20, 1996.
5. Palayoor, S. T., Youmell, M. Y., Calderwood, S. K., Coleman, C. N., and Price, B. D. Constitutive activation of IκB kinase a and NF-κB in prostate cancer cells is inhibited by ibuprofen. Oncogene, *18:* 7389–7394, 1999.
6. Sumitomo, M., Tachibana, M., Nakashima, J., Murai, M., Miyajima, A., Kimura, F., Hayakawa, M., and Nakamura, H. An essential role for nuclear factor κ B in preventing TNF-α-induced cell death in prostate cancer cells. J. Urol., *161:* 674–679, 1999.
7. Muenchen, H. J., Lin, D. L., Walsh, M. A., Keller, E. T., and Pienta, K. J. Tumor necrosis factor-α-induced apoptosis in prostate cancer cells through inhibition of nuclear factor-κB by an IκBα "super-repressor". Clin. Cancer Res., *6:* 1969–1977, 2000.
8. Matsumoto, N., Iinuma, H., Sawa, T., Takeuchi, T., Hirano, S., Yoshioka, T., and Ishizuka, M. Epoxyquinomicins A. B, C and D, new antibiotics from *Amycolatopsis*. II. Effect on type II collagen-induced arthritis in mice. J. Antibiot. (Tokyo), *50:* 906–911, 1997.
9. Erkel, G., Anke, T., and Sterner, O. Inhibition of NF-κB activation by panepoxydone. Biochem. Biophys. Res. Commun., *226:* 214–221, 1996.
10. Matsumoto, N., Ariga, A., To-e, S., Nakamura, H., Agata, N., Hirano, S., Inoue, J., and Umezawa, K. Synthesis of NF-κB activation inhibitors derived from epoxyquinomicin C. Bioorg. Med. Chem. Lett., *10:* 865–869, 2000.
11. Ariga, A., Namekawa, J., Matsumoto, N., Inoue, J., and Umezawa, K. Inhibition of tumor necrosis factor-α-induced nuclear translocation and activation of NF-κB by dehydroxymethylepoxyquinomicin. J. Biol. Chem., *277:* 24625–24630, 2002.
12. Muraki, J., Addonizio, J. C., Choudhury, M. S., Fischer, J., Eshghi, M., Davidian, M. M., Shapiro, L. R., Wilmot, P. L., Nagamatsu, G. R., and Chiao, J. W. Establishment of new human prostatic cancer cell line (JCA-1). Urology, *36:* 79–84, 1990.
13. Nakashima, J., Brookins, J., Beckman, B., and Fisher, J. W. Increased erythropoietin secretion in human hepatoma cells by N6-cyclohexyladenosine. Am. J. Physiol., *261:* C455–460, 1991.
14. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R., and Verma, I. M. Suppression of TNF-α-induced apoptosis by NF-κB. Science (Wash. DC), *274:* 787–789, 1996.
15. Beg, A. A., and Baltimore, D. An essential role for NF-κB in preventing TNF-α-induced cell death. Science (Wash. DC), *274:* 782–784, 1996.
16. Brown, K., Gerstberger, S., Carlson, L., Franzoso, G., and Siebenlist, U. Control of IκB-α proteolysis by site-specific, signal-induced phosphorylation. Science (Wash. DC), *267:* 1485–1488, 1995.
17. Oya, M., Ohtsubo, M., Takayanagi, A., Tachibana, M., Shimizu, N., and Murai, M. Constitutive activation of nuclear factor-κB prevents TRAIL-induced apoptosis in renal cancer cells. Oncogene, *20:* 3888–3896, 2001.
18. Auphan, N., DiDonato, J. A., Rosette, C., Helmberg, A., and Karin, M. Immunosuppression by glucocorticoids: inhibition of NF-κB activity through induction of IκB synthesis. Science (Wash. DC), *270:* 286–290, 1995.
19. Kopp, E., and Ghosh, S. Inhibition of NF-κB by sodium salicylate and aspirin. Science (Wash. DC), *265:* 956–959, 1994.
20. Gehrt, A., Erkel, G., Anke, T., and Sterner, O. Cycloepoxydon, 1-hydroxy-2-hydroxymethyl-3-pent-1-enylbenzene and 1-hydroxy-2-hydroxymethyl-3-pent-1, 3-dienylbenzene, new inhibitors of eukaryotic signal transduction. J. Antibiot. (Tokyo), *51:* 455–463, 1998.
21. Pahl, H. L., Krauss, B., Schulze-Osthoff, K., Decker, T., Traenckner, E. B., Vogt, M., Myers, C., Parks, T., Warring, P., Muhlbacher, A., Czernilofsky, A. P., and Baeuerle, P. A. The immunosuppressive fungal metabolite gliotoxin specifically inhibits transcription factor NF-κB. J. Exp. Med., *183:* 1829–1840, 1996.
22. van Bokhoven, A., Varella-Garcia, M., Korch, C., and Miller, G. J. TSU-Pr1 and JCA-1 cells are derivatives of T24 bladder carcinoma cells and are not of prostatic origin. Cancer Res., *61:* 6340–6344, 2001.