ONCOLOGY REPORTS 33: 2940-2946, 2015

Tetraarsenic hexoxide demonstrates anticancer activity at least in part through suppression of NF-κB activity in SW620 human colon cancer cells

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Received January 30, 2015; Accepted March 5, 2015

DOI: 10.3892/or.2015.3890

Abstract. Tetraarsenic hexoxide (As_4O_6) has been used in Korean traditional medicine for the treatment of cancer since the late 1980's, and arsenic trioxide (As_2O_3) is currently used as a chemotherapeutic agent. Previous studies suggest that the As_4O_6-induced cell death pathway is different from that of As_2O_3 and its mechanism of anticancer activity remains unclear. Nuclear factor (NF)-κB is a well-known transcription factor involved in cell proliferation, invasion and metastasis. Hence, in the present study, we investigated the effects of As_4O_6 on NF-κB activity and NF-κB-regulated gene expression *in vitro* and *in vivo*. The cytotoxicity assay revealed that As_4O_6 inhibited the growth of SW620 cells in a dose-dependent manner, and the half maximal inhibitory concentration (IC_{50}) was ~1 μM after a 48 h treatment. As_4O_6 suppressed NF-κB activation and suppressed inhibitory κBα (IκBα) phosphorylation stimulated by tumor necrosis factor (TNF). As_4O_6 also suppressed downstream NF-κB-regulated proteins involved in cancer anti-apoptosis, proliferation, invasion and metastasis. In addition, As_4O_6 marginally suppressed tumor growth and the anti-NF-κB activity was confirmed using an *in vivo* xenograft mouse model in which animals were injected with SW620 cells. The present study provides evidence that As_4O_6 has anticancer properties through suppression of NF-κB activity and NF-κB-mediated cellular responses.

Introduction

Colon cancer is one of the most common cancers in the world (1). Regarding treatment, surgical resection is frequently limited due to metastasis such as in most other cancers. Although several chemotherapeutic drugs are available for the treatment of metastatic lesions, the toxic effects are serious. Recently, with the advancement in science, the life-span has been increasing, and the elderly population with cancer is also increasing. However, these patients cannot tolerate the cytotoxic effects of chemotherapies. Therefore, new treatment strategies are required for elderly patients. Arsenic trioxide (As_2O_3) had been used in Chinese medicine for cancer treatment, and is now used as a standard treatment for refractory acute promyelocytic leukemia (2,3). Several clinical trials have been performed in certain types of solid cancers (4,5), yet they failed to prove clinical efficacy due to high toxicities (6,7). Tetraarsenic hexoxide (As_4O_6) has been used as a Korean folk remedy for the management of cancer since the late 1980's and shows no serious toxicities. However, little research regarding the anticancer effects of As_4O_6 has been conducted even though previous studies have shown that the anticancer effects of As_4O_6 are more potent than those of As_2O_3 in human cancer cells *in vitro*, and that the signaling pathways of As_4O_6-induced cell death are different from those of As_2O_3 (8,9). We previously demonstrated that As_4O_6 has synergistic effects with tumor necrosis factor (TNF). TNF is known as a stimulator of nuclear factor (NF)-κB and NF-κB is a transcription factor closely linked to cell survival, proliferation and metastasis (10). In the present study, we explored the anticancer effects of As_4O_6 with special focus on the NF-κB pathway, on NF-κB-regulated gene products and on NF-κB-mediated cellular responses.

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Key words: tetraarsenic hexoxide, nuclear factor-κB, anticancer effects, colon cancer

Materials and methods

Cells and reagents. SW620 human colon cancer cells purchased from the American Type Culture Collection (Rockville, MD, USA) were cultured in RPMI-1640 medium (Invitrogen Corp., Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco-BRL, Grand Island, NY, USA), 1 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C in a humidified atmosphere of 95% air and 5% CO_2. As_4O_6 was provided by the Chonjisan Institute (Seoul, Korea). Antibodies against NF-κB (p65), cyclin D1, Bcl-2, Bcl-xL, XIAP, cIAP-1, cIAP-2, MMP-2, MMP-9, VEGF, p-NF-κB, transglutaminase 2 (TG-2), Ki-67 and CD34 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). An antibody against β-actin was from Sigma (Beverly, MA, USA). Peroxidase-labeled donkey anti-rabbit and sheep anti-mouse immunoglobulins, and an enhanced chemiluminescence (ECL) kit were purchased from Amersham (Arlington Heights, IL, USA). All other chemicals not specifically cited here were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All of these solutions were stored at -20˚C. Stock solutions of 4',6-diamidino-2-phenylindole (DAPI) (100 μg/ml) and propidium iodide (PI; 1 mg/ml) were prepared in phosphate-buffered saline (PBS).

Cell viability assay. For the cell viability assay, the cells were seeded onto 24-well plates at a concentration of $5x10^5$ cells/ml, and then treated with the indicated concentration of As_4O_6 for 24 or 48 h. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/ml) was subsequently added to each well. After 3 h of additional incubation, 100 μl of a solution containing 10% SDS (pH 4.8) plus 0.01 N HCl was added to dissolve the crystals. The absorption values at 570 nm were determined with an ELISA plate reader.

Western blotting. Total cell lysates were obtained using lysis buffer containing 0.5% SDS, 1% NP-40, 1% sodium deoxycholate, 150 mM NaCl, 50 mM Tris-Cl (pH 7.5) and protease inhibitors. The concentrations of cell lysate proteins were determined by the Bradford protein assay (Bio-Rad Laboratories, Richmond, CA, USA) using bovine serum albumin as the standard. To determine the protein expression of NF-κB in the cytoplasm and the nuclei, we prepared separate extracts. The cells were washed with ice-cold PBS (pH 7.4) and lysed in buffer A [10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol (DTT), 5 μM leupeptin, 2 μM pepstatin A, 1 μM aprotinin and 20 μM phenylmethylsulfonyl fluoride] by repeated freezing and thawing. Nuclear and cytoplasmic fractions were separated by centrifugation at 1,000 x g for 20 min. The cytoplasmic extract (supernatant) was obtained. The pellets were washed with buffer A, and resuspended in buffer B [10 mM Tris-Cl (pH 7.5), 0.5% deoxycholate, 1% NP-40, 5 mM EDTA, 0.5 mM DTT, 5 μM leupeptin, 2 μM pepstatin A, 1 μM aprotinin and 20 μM phenylmethylsulfonyl fluoride]. The suspension was agitated for 30 min at 4˚C and centrifuged at 10,000 x g for 20 min. The supernatant fraction containing nuclear proteins was collected. Molecular mass markers for proteins were obtained from Pharmacia Biotech (Saclay, France). Thirty micrograms of the lysate proteins were resolved by electrophoresis, electrotransferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA), and then incubated with primary antibodies followed by a secondary antibody conjugated to peroxidase. Blots were developed with an ECL detection system.

Immunocytochemistry. The cells were placed on coverslips coated with poly-L-lysine (1 mg/ml) in 6-well plates. They were fixed in 4% paraformaldehyde for 10 min followed by 1.0% H_2O_2/0.1 M PBS treatment for 30 min after washing twice in PBS. Then, cells were treated with 0.3% Triton/0.1 M PBS for 5 min and then washed twice in buffered saline. They were incubated in 5% serum solution for 30 min at room temperature and then serum solution was removed with suction. The cells were incubated in buffered saline with a 1:50 dilution of primary antibodies for p65 NF-κB (Santa Cruz Biotechnology, Inc.) for 2 h and then washed in buffered saline three times for 10 min each at room temperature. They were incubated in buffered saline with a 1:250 dilution of biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA, USA). Positive staining was visualized with diaminobenzidine, followed by a light hematoxylin counter-staining.

Transfection. NF-κB-luciferase constructs (consensus NF-κB binding sequence was cloned into the pGL3 basic luciferase expression vector) were kindly provided by Dr G. Koretzky (University of Pennsylvania). Transient transfection was performed using Lipofectamine (Gibco-BRL) according to the manufacturer's protocol.

Luciferase assay. After experimental treatments, the cells were washed twice with cold PBS, lysed in a passive lysis buffer provided in the Dual-Luciferase kit (Promega, Madison, WI, USA), and assayed for luciferase activity using a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA, USA) according to the manufacturer's protocol. Data are presented as a ratio between firefly and *Renilla* luciferase activities.

Generation of xenograft tumors and immunohistochemical staining. All animal procedures were performed in accordance with a protocol approved by the Ethics Committee for Animal Experimentation, Gyeongsang National University. We followed animal science guidelines for animal experimentation. Xenograft tumors were generated by subcutaneous injection of SW620 cells, as described elsewhere (11). Briefly, nude mice were injected in a single dorsal flank site with $5x10^7$ SW620 cells (n=12 mice) in 100 μl of PBS. Injection of these cells into nude mice induced exponentially growing tumors. When tumors reached a volume of 50-100 mm^3 (termed day 0 for our experiments), the mice were treated intraperitoneally with vehicle (1 μl of normal saline) or As_4O_6 at 5 mg/kg once a day for 12 days. Tumor size was measured every 3-4 days, and tumor growth was quantified by measuring the tumors in two dimensions. Volumes were calculated by the formula: 0.5 x a x b, where a and b are the longest and the greatest perpendicular diameters, respectively. Tumor volumes were expressed as the mean and 95% confidence interval (CI) and expressed as relative change vs. time. Histopathologic evidence of pulmonary toxicity (i.e., edema or inflammation of the bronchial epithelium and alveoli), inflammation or injury in other organs, such as liver, and kidney were evaluated by a pathologist. Tumors were fixed in 10% buffered formalin, embedded in paraffin, and sectioned for hematoxylin and eosin (H&E) and immunohistochemical staining. Immunohistochemical staining for p-NF-κB, TG, Ki-67 and tumor vessel density was performed as previously described (12).

Statistical analysis. Each experiment was performed in triplicate. The results are expressed as means ± SD. Significant differences were determined using the one-way ANOVA with *post-hoc* Neuman-Keuls test in the case of at least three treatment groups and Student's t-test for two group comparison. Statistical significance was defined as $P<0.05$.

Results

As_4O_6 suppresses cell proliferation of SW620 human colon cancer cells in a dose-dependent manner. To investigate the antitumor activity of As_4O_6 in SW620 cells, the cells were treated for 24 and 48 h with various concentrations of As_4O_6 (0.1-5 μM), and the cell growth was assessed by MTT assay. The MTT assay revealed that As_4O_6 inhibited the growth of SW620 cells in a dose-dependent manner at 24 and 48 h. As_4O_6 had a strong inhibitory effect after 48 h of treatment and the half maximal inhibitory concentration (IC_{50}) was ~1 μM (Fig. 1A). Next, we assessed the changes in cellular morphology of the As_4O_6-treated cells under microscopy. The light microscopy results revealed that cell shrinkage and cytoplasmic blebs were observed after 24 and 48 h of incubation (Fig. 1B).

As_4O_6 suppresses NF-κB activity at least in part through inhibition of IκBα phosphorylation. To determine whether As_4O_6 inhibits *NF-κB* activity of SW620 cells, we used western blotting, immunohistocytochemistry and luciferase assay. Under resting conditions, NF-κB mostly consists of a heterotrimer of p50, p65 and inhibitory κBα (IκBα) in the cytoplasm; when activated, the heterodimer of p50 and p65 is translocated into the nucleus after separating from p-IκBα. Hence, we performed western blot analysis, which revealed that As_4O_6 reduced both the translocation of NF-κB into the nucleus and the levels of NF-κB in the cytoplasm (Fig. 2A). One advantage of immunohistochemistry is the ability to confirm NF-κB (p65) translocation into the nucleus on activation. As expected, TNF enhanced the NF-κB translocation into the nucleus and As_4O_6 inhibited the TNF-induced NF-κB activation (Fig. 2B). To confirm the effects of As_4O_6 on NF-κB activity, we performed a luciferase assay. As shown in Fig. 2C, the NF-κB gene was successfully transfected into the cells and the NF-κB-luciferase activity was augmented by TNF. The NF-κB-luciferase activity induced by TNF was inhibited by As_4O_6 (Fig. 2C). As mentioned, NF-κB activation is required for the degradation of IκBα through phosphorylation by kinases. We also tested whether As_4O_6 suppressed TNF-induced phosphorylation of IκBα. Western blot analysis revealed that As_4O_6 prevented TNF-induced IκBα phosphorylation (Fig. 2D). This result suggested that As_4O_6 suppressed NF-κB activity at least in part through inhibition of IκBα phosphorylation.

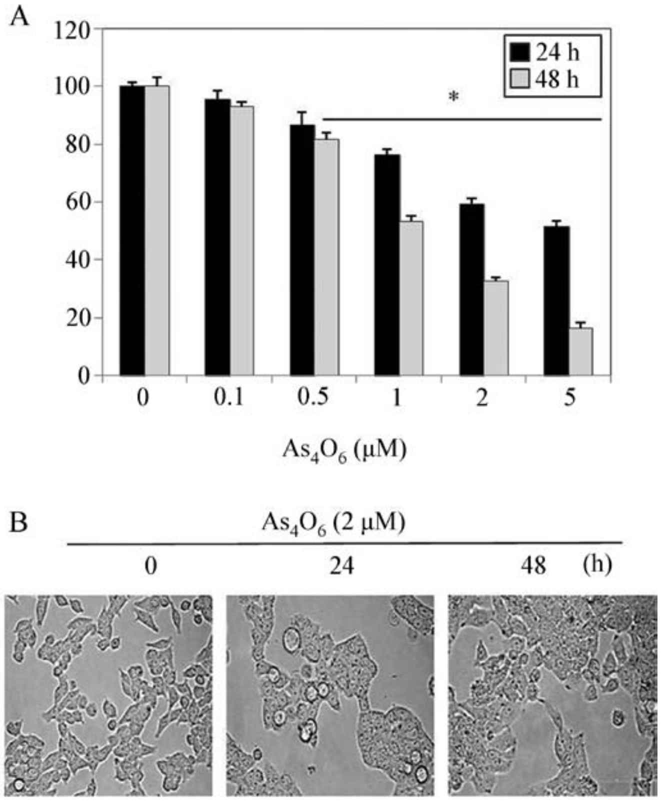

Figure 1. Inhibition of cell growth by As_4O_6 in SW620 cells. The cells were seeded at a density of $5x10^4$ cells/ml. The inhibition of cell growth was measured by MTT assay. (A) Cells were treated with the indicated concentrations of As_4O_6 for 24 and 48 h. The growth inhibition and cytotoxicity of As_4O_6 was executed in a dose-dependent manner. (B) Cells were photographed under an inverted microscope (original magnification, x200). The data are shown as means ± SD of three independent experiments. $^*P<0.05$ between the treated and control group. As_4O_6, tetraarsenic hexoxide.

As_4O_6 suppresses NF-κB-regulated proteins involved in anti-apoptosis, proliferation, invasion and angiogenesis. NF-κB activation leads to activation of several genes involved in anti-apoptosis, proliferation, invasion and angiogenesis in cancer. NF-κB regulates expression of anti-apoptotic proteins (c-IAP1/2, XIAP and Bcl-xL) (13), cyclin D1 for cell proliferation (14), MMP-2, MMP-9 for invasion and VEGF for angiogenesis of cancer (13,15). Hence, we investigated the effect of As_4O_6 on these molecules. Western blot analysis revealed that As_4O_6 suppressed the protein expression of XIAP, Bcl-2, Bcl-xL, cIAP-1, cyclin D1, MMP-2, MMP-9 and VEGF in a dose-and time-dependent manner (Fig. 3). These findings revealed that As_4O_6 suppressed the NF-κB-mediated cellular responses regarding cancer apoptosis, proliferation, invasion and angiogenesis in the SW620 cells.

As_4O_6 marginally suppresses the tumor growth of SW620 cells. Next, we evaluated the effect of As_4O_6 treatment on the growth of SW620 cells (Fig. 4). Tumor growth was marginally suppressed by As_4O_6 treatment throughout the 12-day treatment regimen, indicating the potent therapeutic efficacy of As_4O_6 in SW620 cancer cells (Fig. 4A). The volume of the control SW620 xenografts was 798 mm^3 and that of the xenografts treated with As_4O_6 at 5 mg/kg was 115.9 mm^3

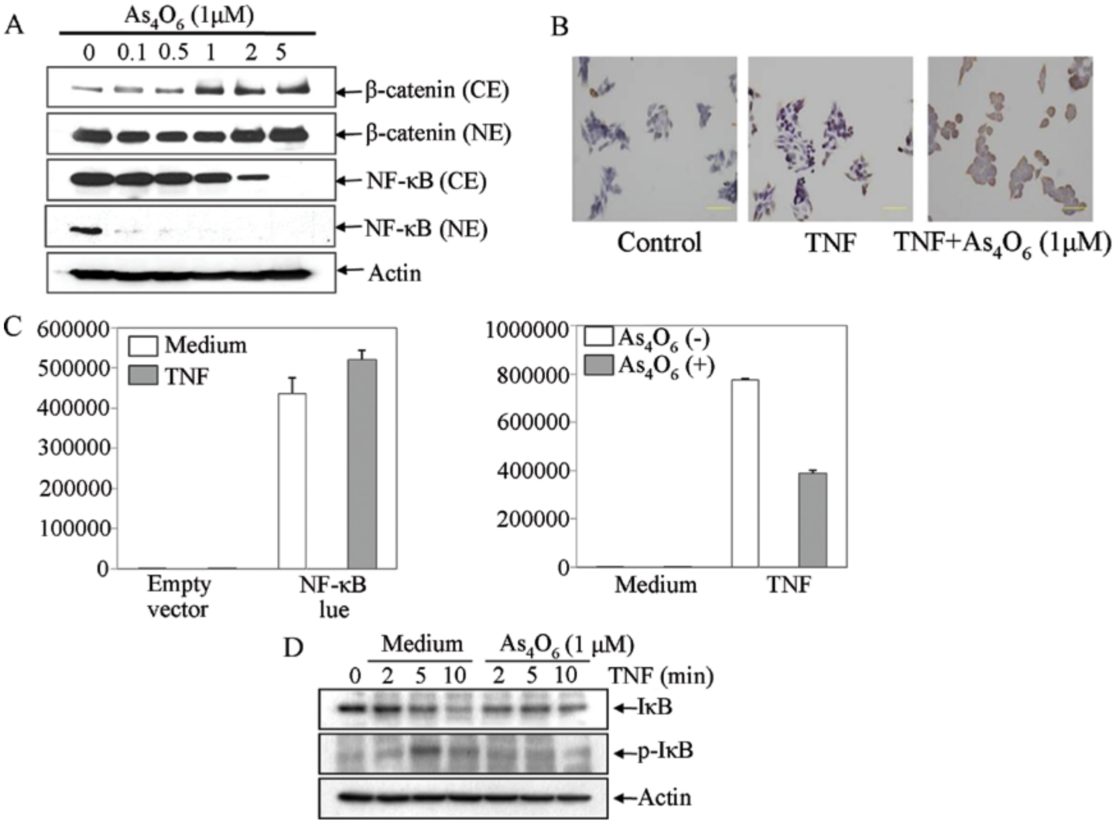

Figure 2. Effects of As_4O_6 on NF-κB and the IκBα phosphorylation. (A) Inhibitory effects of As_4O_6 on TNF-induced NF-κB translocation into the nucleus. Cells were treated with As_4O_6 (1 μM) for 48 h at the indicated concentrations. After treatment, nuclear (NE) and cytoplasmic (CE) fractions were extracted from total cell lysates and protein levels were determined by western blot analysis. (B) Immunocytochemical analysis of NF-κB (p65) localization in the SW620 cells. Cells were pretreated with As_4O_6 (1 μM) or 0.1% DMSO (vehicle control) for 24 h and then treated with TNF (10 ng/ml) for 30 min (x400, magnification; scale bar, 50 μm). (C) Cells were transfected with an empty vector or 1 μg of NF-κB-luciferase (lue). The cells were allowed to recover for 24 h and then treated with 10 ng/ml of TNF with/without a 1-h pretreatment of As_4O_6 (1 μM). The cells were harvested 1 h post-treatment with TNF-α and luciferase activities are presented as fold-activation relative to that of the untreated control. (D) Inhibitory effects of 1 μM on IκBα phosphorylation. Cells were pretreated with As_4O_6 (1 μM) for 1 h and then treated with TNF (10 ng/ml) for the indicated times. Each bar graph represents the mean ± SD of three independent experiments. $^*P<0.05$ between the treated and control group. As_4O_6, tetraarsenic hexoxide; NF-κB, nuclear factor-κB; TNF, tumor necrosis factor; DMSO, dimethylsulfoxide.

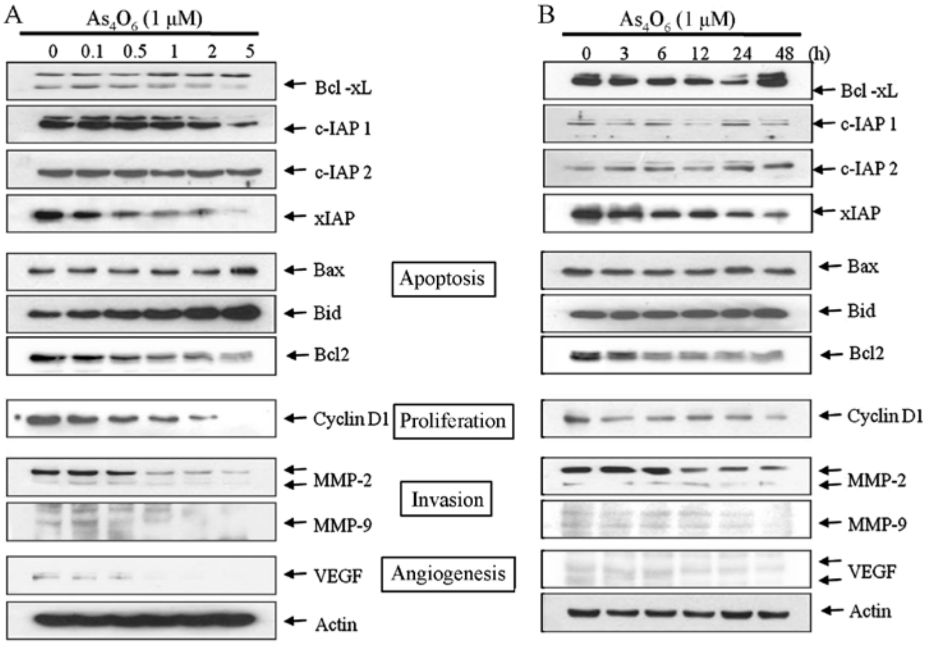

Figure 3. Effects of As_4O_6 on NF-κB-regulated proteins involved in anti-apoptosis, proliferation, invasion and angiogensis. (A) SW620 cells ($5x10^4$) were either left untreated or pretreated with As_4O_6 at the indicated doses for 48 h or (B) pretreated with As_4O_6 at 1 μM for the indicated times and then whole-cell extracts were prepared. Whole-cell lysate (30 μg) was analyzed by western blotting using antibodies against various NF-κB-regulated proteins involved in cancer cell anti-apoptosis, proliferation and invasion and angiogenesis. As_4O_6, tetraarsenic hexoxide; NF-κB, nuclear factor-κB.

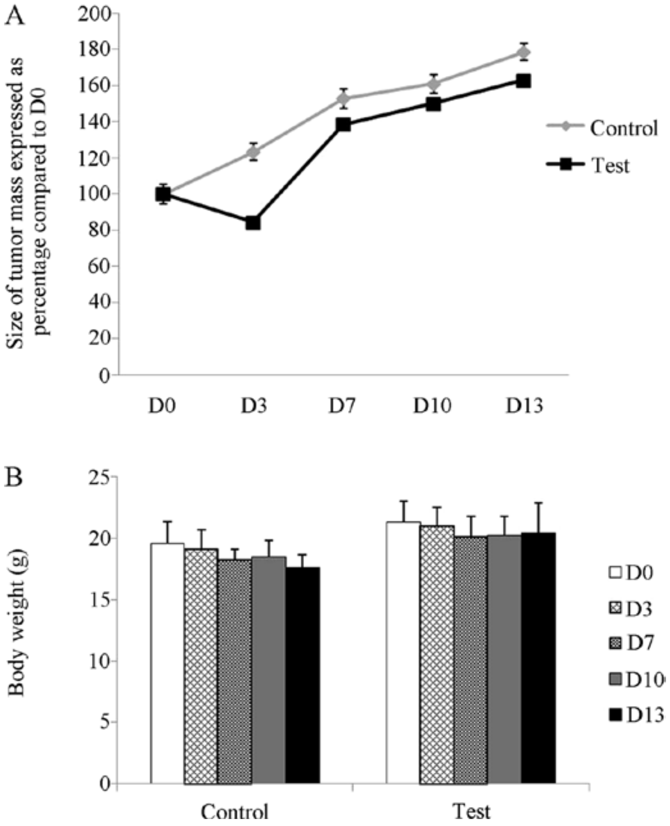

Figure 4. Effects of As_4O_6 in the SW620 xenograft model. Nude mice were injected at a single dorsal flank site with 5x10^7 SW620 cells (n=12 mice) in 100 μl of phosphate-buffered saline (PBS). Once the tumors reached a volume of 50-100 mm^3, the mice were treated with vehicle (1 μl of normal saline) or As_4O_6 at 5 mg/kg once a day for 12 days. Tumor volumes and body weights were measured every 2-4 days. (A) Mean tumor volumes expressed as a percentage compared to day 0 treatment were plotted against the day of treatment. (B) Body weight of control and As_4O_6-treated groups at indicated day intervals. Each bar graph represents the mean ± SD of three independent experiments. $^*P<0.05$ between the treated and the untreated control group. As_4O_6, tetraarsenic hexoxide.

(difference, 682.1 mm^3; 95% CI, 480.4-883.9 mm^3; $P<0.001$). Also, there were no significant difference in body weight between the control and treatment groups (Fig. 4B).

As_4O_6 suppresses NF-κB activity and NF-κB-mediated cellular phenotype such as cancer proliferation and angiogenesis in the in vivo xenograft mouse model. We further investigated the *in vivo* effect of As_4O_6 treatment on NF-κB activity and NF-κB-regulated proteins in the SW620 xenograft tumors. Immunohistochemical studies revealed that the expression of p-NF-κB in the tumors from the As_4O_6-treated mice was lower than that in the control tumors from the untreated mice (Fig. 5). Here, we also tested TG-2 since TG-2 expression has a good correlation with NF-κB activity (16), and a difference in p-NF-κB expression is not easily observed. The result indicated that As_4O_6 significantly suppressed TG-2 expression. In addition As_4O_6 also clearly suppressed CD34, a protein which is involved in angiogenesis and Ki-67, a nuclear protein that is associated with cellular proliferation. These findings were consistent with p-NF-κB expression and suggest that As_4O_6 may suppress NF-κB activity and NF-κB-regulated cellular phenotype.

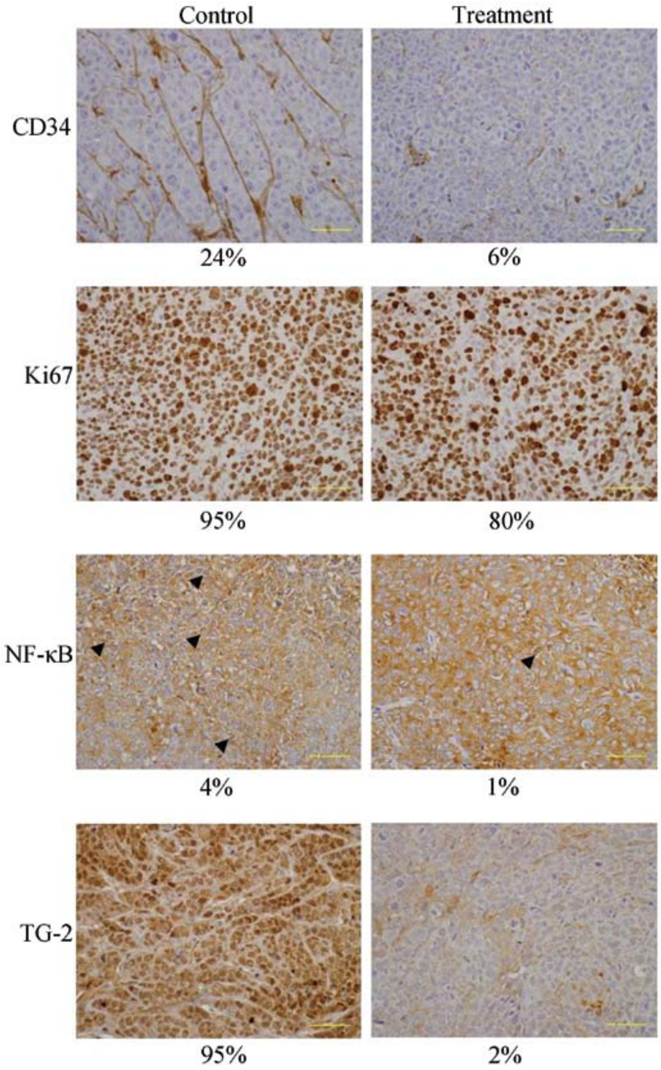

Figure 5. H&E staining of tumor tissues from the SW620 xenografts after As_4O_6 treatment. Nude mice were injected at a single dorsal flank site with 5x10^7 SW620 cells (n=12 mice) in 100 μl of phosphate-buffered saline (PBS). Once the tumors reached a volume of 50-100 mm^3, the mice were treated with vehicle (1 μl of normal saline) or As_4O_6 at 5 mg/kg once a day for 12 days. H&E and immunohistochemical staining of tumors evaluated for CD34, p-NF-κB, TG-2, Ki-67 and tumor vessel density (magnification, x200). H&E, hematoxylin and eosin; As_4O_6, tetraarsenic hexoxide.

Discussion

The present study was designed to investigate the anticancer effects of As_4O_6 with special focus on the NF-κB pathway, and NF-κB-regulated gene products, in *in vitro* and *in vivo* models. We found that As_4O_6 inhibited the growth of SW620 cells in a dose-dependent manner at 24 and 48 h. Furthermore, As_4O_6 inhibited NF-κB activity and NF-κB-regulated proteins involved in anti-apoptosis, cell proliferation, invasion and angiogenesis. Even though this finding is novel for As_4O_6, there is previous supporting evidence showing that arsenic trioxide (As_2O_3) suppresses NF-κB-mediated cellular activities (17). NF-κB is a well-known transcription factor involved in cancer proliferation, invasion, metastasis and drug resistance. We found that As_4O_6 suppressed MMP-2 and MMP-9 activity. MMP-2 and MMP-9 are key molecules in cancer cell invasion (18,19) which have been used as targets

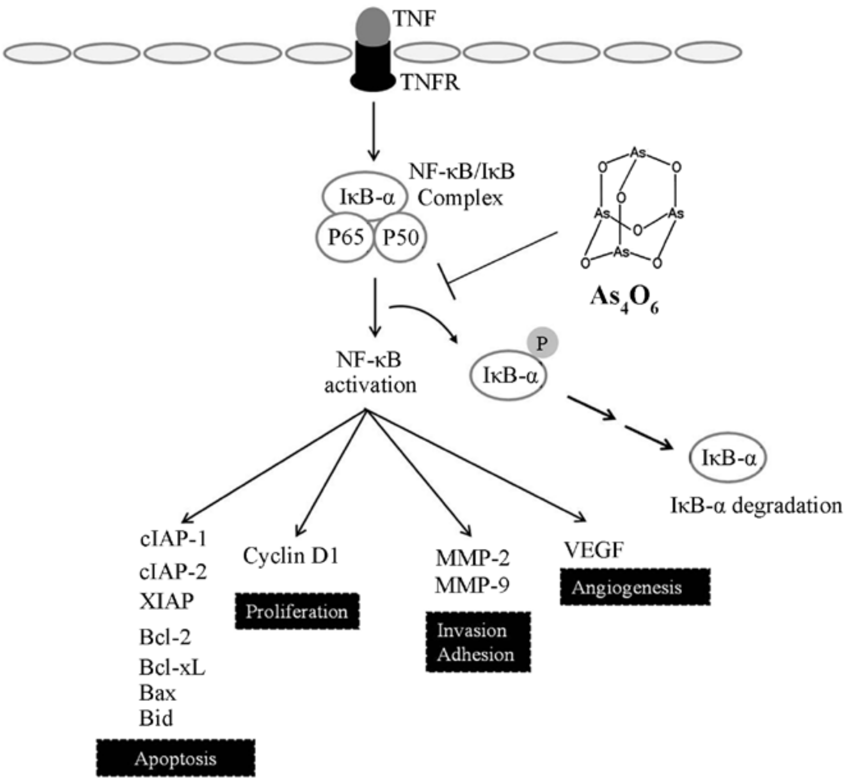

Figure 6. Schematic representation of the anticancer effects of As_4O_6 on SW620 human colon cancer cells. As_4O_6 suppressed the invasive effects of SW620 cells by suppression of NF-κB through inhibition of IκB phosphorylation stimulated by TNF. In addition, TNF participated in induction of NF-κB-regulated proteins involved in cancer cell proliferation (cyclin D1), anti-apoptosis (XIAP, IAP1, IAP2, Bcl-xL, Bcl-2, Bax and Bid), and invasion and angiogenesis (MMP-2, MMP-9 and VEGF). Taken together, the present study suggests that As_4O_6 has anticancer properties through suppression of NF-κB activity and NF-κB-mediated cellular responses. As_4O_6, tetraarsenic hexoxide; NF-κB, nuclear factor-κB.

for drug development against cancer invasion (20). We also found that As_4O_6 suppressed cyclin D1 which is associated with cancer cell proliferation (13,14), and XIAP, Bcl-2, Bcl-xL and cIAP-1 that are involved in cancer cell survival and drug resistance (13). In addition, the role of VEGF in the angiogenesis of cancer is well known (21). All of these gene products are known to be regulated by NF-κB (13,15). Here, we used TNF to clearly demonstrate that As_4O_6 inhibits NF-κB. Plasma TNF is usually increased in patients with advanced and metastatic cancers (22). The pathophysiological relevance between TNF and NF-κB activation in advanced and metastatic cancers suggests that the use of TNF is also similar to the cancer environment in the human body. IκBα is the best-studied and a major IκB protein of the IκB family. When activated by signals, the IκB kinase phosphorylates two serine residues located in an IκBα regulatory domain. When IκBα is phosphorylated at serines 32 and 36, IκBα is degraded by ubiquitination (23). Here, we found that As_4O_6 suppressed phosphorylation of IκBα induced by TNF. This finding suggests that the anti-NF-κB activities of As_4O_6 are contributed to suppression of IκBα phosphorylation. In addition, we demonstrated that As_4O_6 inhibited NF-κB activity in an *in vivo* animal model even though the anticancer effects were marginal. One weak point is that although As_4O_6 suppressed the whole expression of NF-κB (Fig. 2A), we could not exactly elucidate the mechanisms. We also found that As_4O_6 suppressed the whole expression of NF-κB (data not shown). We need to further investigate this mechanism.

In conclusion, the present study demonstrated that As_4O_6 exerts anticancer effects by suppressing NF-κB and NF-κB-regulated genes involved in anti-apoptosis, proliferation, invasion and angiogenesis in cancer (Fig. 6). The present study provides evidence that As_4O_6 may have anticancer effects on human colon cancer.

Acknowledgements

This study was supported by a grant from the National Research Foundation of Korea (NRF) funded by the Korea government (MEST) (no. 2014-012154).

References

1. El-Serag HB and Mason AC: Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 340: 745-750, 1999.
2. Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY, Zhu J, Tang W, Sun GL, Yang KQ, *et al*: Use of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL). II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 89: 3354-3360, 1997.
3. Niu C, Yan H, Yu T, Sun HP, Liu JX, Li XS, Wu W, Zhang FQ, Chen Y, Zhou L, *et al*: Studies on treatment of acute promyelocytic leukemia with arsenic trioxide: Remission induction, follow-up, and molecular monitoring in 11 newly diagnosed and 47 relapsed acute promyelocytic leukemia patients. Blood 94: 3315-3324, 1999.
4. Munshi NC, Tricot G, Desikan R, Badros A, Zangari M, Toor A, Morris C, Anaissie E and Barlogie B: Clinical activity of arsenic trioxide for the treatment of multiple myeloma. Leukemia 16: 1835-1837, 2002.

5. Lin YC, Li DR and Lin W: Relationship between radiotherapy enhancing effect of arsenic trioxide and the proliferation and apoptosis of related protein in nasopharyngeal carcinoma patients. Zhongguo Zhong Xi Yi Jie He Za Zhi 27: 704-707, 2007 (In Chinese).
6. Welch JS, Klco JM, Gao F, Procknow E, Uy GL, Stockerl-Goldstein KE, Abboud CN, Westervelt P, DiPersio JF, Hassan A, *et al*: Combination decitabine, arsenic trioxide, and ascorbic acid for the treatment of myelodysplastic syndrome and acute myeloid leukemia: A phase I study. Am J Hematol 86: 796-800, 2011.
7. Beer TM, Tangen CM, Nichols CR, Margolin KA, Dreicer R, Stephenson WT, Quinn DI, Raghavan D and Crawford ED: Southwest Oncology Group phase II study of arsenic trioxide in patients with refractory germ cell malignancies. Cancer 106: 2624-2629, 2006.
8. Chang HS, Bae SM, Kim YW, Kwak SY, Min HJ, Bae IJ, Lee YJ, Shin JC, Kim CK and Ahn WS: Comparison of diarsenic oxide and tetraarsenic oxide on anticancer effects: Relation to the apoptosis molecular pathway. Int J Oncol 30: 1129-1135, 2007.
9. Han MH, Lee WS, Lu JN, Yun JW, Kim G, Jung JM, Kim GY, Lee SJ, Kim WJ and Choi YH: Tetraarsenic hexoxide induces Beclin-1-induced autophagic cell death as well as caspase-dependent apoptosis in U937 human leukemic cells. Evid Based Complement Alternat Med 2012: 201414, 2012.
10. Guttridge DC, Albanese C, Reuther JY, Pestell RG and Baldwin AS Jr: NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. Mol Cell Biol 19: 5785-5799, 1999.
11. Lee HY, Moon H, Chun KH, Chang YS, Hassan K, Ji L, Lotan R, Khuri FR and Hong WK: Effects of insulin-like growth factor binding protein-3 and farnesyltransferase inhibitor SCH66336 on Akt expression and apoptosis in non-small-cell lung cancer cells. J Natl Cancer Inst 96: 1536-1548, 2004.
12. Jang JS, Lee WS, Lee JS, Kim HW, Ko GH and Ha WS: The expression of thymidine phosphorylase in cancer-infiltrating inflammatory cells in stomach cancer. J Korean Med Sci 22 (Suppl 22): S109-S114, 2007.
13. Aggarwal BB: Nuclear factor-kappaB: The enemy within. Cancer Cell 6: 203-208, 2004.
14. Motokura T and Arnold A: PRAD1/cyclin D1 proto-oncogene: Genomic organization, 5' DNA sequence, and sequence of a tumor-specific rearrangement breakpoint. Genes Chromosomes Cancer 7: 89-95, 1993.
15. Gilmore TD: Introduction to NF-kappaB: Players, pathways, perspectives. Oncogene 25: 6680-6684, 2006.
16. Kim DS, Park SS, Nam BH, Kim IH and Kim SY: Reversal of drug resistance in breast cancer cells by transglutaminase 2 inhibition and nuclear factor-kappaB inactivation. Cancer Res 66: 10936-10943, 2006.
17. Kerbauy DM, Lesnikov V, Abbasi N, Seal S, Scott B and Deeg HJ: NF-kappaB and FLIP in arsenic trioxide (ATO)-induced apoptosis in myelodysplastic syndromes (MDSs). Blood 106: 3917-3925, 2005.
18. Davies B, Waxman J, Wasan H, Abel P, Williams G, Krausz T, Neal D, Thomas D, Hanby A and Balkwill F: Levels of matrix metalloproteases in bladder cancer correlate with tumor grade and invasion. Cancer Res 53: 5365-5369, 1993.
19. Bogenrieder T and Herlyn M: Axis of evil: Molecular mechanisms of cancer metastasis. Oncogene 22: 6524-6536, 2003.
20. Vihinen P and Kähäri VM: Matrix metalloproteinases in cancer: Prognostic markers and therapeutic targets. Int J Cancer 99: 157-166, 2002.
21. Nishida N, Yano H, Nishida T, Kamura T and Kojiro M: Angiogenesis in cancer. Vasc Health Risk Manag 2: 213-219, 2006.
22. Correia M, Cravo M, Marques-Vidal P, Grimble R, Dias-Pereira A, Faias S and Nobre-Leitão C: Serum concentrations of TNF-alpha as a surrogate marker for malnutrition and worse quality of life in patients with gastric cancer. Clin Nutr 26: 728-735, 2007.
23. Chen ZJ, Parent L and Maniatis T: Site-specific phosphorylation of IkappaBalpha by a novel ubiquitination-dependent protein kinase activity. Cell 84: 853-862, 1996.