

Laboratory Investigation

Dae Won Kim, M.D.
 Song Ho Ahn, M.D.
 Tae Young Kim, M.D.

Department of Neurosurgery
 School of Medicine
 Wonkwang University
 Iksan, Korea

Enhancement of Arsenic Trioxide (As_2O_3)-Mediated Apoptosis Using Berberine in Human Neuroblastoma SH-SY5Y Cells

Objective : Arsenic trioxide (As_2O_3) has been used as an anticancer agent in traditional Chinese medicine for thousand years and berberine is an isoquinoline alkaloid present that has indicated significant antimicrobial activity. We have examined the combined anticancer effects of As_2O_3 and berberine against the human neuroblastoma (HNB) SH-SY5Y cells *in vitro*, and to elucidate underlying molecular mechanism.

Methods : HNB SH-SY5Y cells were treated with 2 μM As_2O_3 and 75 $\mu g/ml$ berberine, and their survival, cell death mechanism as well as synergistic cytotoxic effects were estimated by using MTT assay, DAPI staining, agarose gel electrophoresis, flow cytometric analysis, and western blot analysis.

Results : The combined treatment of two drugs also markedly decreased cell viability. The cytotoxic effects of two drugs were revealed as apoptosis characterized by chromatin condensation, DNA fragmentation, and the loss of mitochondrial membrane potential. The apoptotic cytotoxicity was accompanied by activation of caspase-3 protease as well as decreased the expression of Bcl-2, Bid, and Bcl-x/L. In addition, the cells treated with combination of two drugs also showed significantly increased intracellular reactive oxygen species levels and lipid peroxidation compared to cells As_2O_3 or berberine only.

Conclusion : Combined treatment of As_2O_3 with berberine induced activation of apoptotic signaling pathways in HNB SH-SY5Y cells. These results suggest that the possibility of the combined treatment of two chemotherapeutic agents with low concentration improving cytotoxic effect for cancer cells with minimal side effects.

KEY WORDS : Human neuroblastoma SH-SY5Y cells · Arsenic trioxide · Berberine · Apoptosis.

INTRODUCTION

Neuroblastoma is the most common extracranial neoplasm of childhood derived from precursor of immature cells of the sympathetic nervous system. Neuroblastomas show a very complex biological and tremendous clinical heterogeneity. The same remarkable cure rate that has been achieved in most other childhood malignancies has not yet occurred in this tumor. The important factors that determine the prognosis and therapeutic modality are patient age, stage, molecular defect and pathology. The majority of the neuroblastomas in children over 1 year age are aggressive metastatic tumors with poor clinical outcome despite intensive multimodal therapy including surgery, irradiation and chemotherapy.

Arsenic trioxide (As_2O_3) has been used as an anticancer agent in traditional Chinese medicine for thousand years. Recently, As_2O_3 was approved by the Food and Drug Administration for use in the treatment of relapsed/refractory acute promyelocytic leukemia, head and neck cancer, neuroblastoma, etc.^{4,9,15}. The mechanism of action is apoptosis induced by making reactive oxygen species (ROS), change of mitochondrial membrane potential (MMP), decreasing Bcl-2 protein expression, and caspases activation. Also, arsenic acid induces DNA damage and regression cell cycle by effect on G0 or G2/M phase^{3,24}.

Berberine, a main component of *Coptidis Rhizoma*, is a type of alkaloid that was initially isolated from Chinese herbal medicine. Berberine has been used in treatments of various diseases, because of its antibacterial, anti-inflammatory, and anticancer effect^{1,2,8,16}. In USA, it is also used as prostatic cancer drug, the brand name of PC-SPECS[®]. Berberine exhibits the ability to induce apoptosis in human cancer cells. Apoptosis is triggered by DNA fragmentation of S-phase cells with specific inhibition of DNA topoisomerase I and II in biochemical system by berberine^{15,20}.

One of the primary issues for cancer chemotherapy is how to kill cancer cells selectively without

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 • Address for reprints :
 Tae Young Kim, M.D.
 Department of Neurosurgery
 School of Medicine
 Wonkwang University
 344-2 Sinyong-dong
 Iksan 570-711, Korea
 Tel : +82-63-859-1462
 Fax : +82-63-852-2606
 E-mail : tykim@wonkwang.ac.kr

damaging normal cells. In particular, combination treatment of two chemotherapeutic agents with low concentration has been reported to improve cytotoxic effect for cancer cells with minimal side effects^{2,10,17,19,22,27}. In this study, we examined the apoptotic effects of combination treatment of berberine and As₂O₃ on HNB SH-SY5Y cell line.

MATERIALS AND METHODS

Chemical reagents

Minimum Essential Medium (MEM) and F12 medium were purchased from Gibco Life Technologies (Gaithersburg, MD, USA). Fetal bovine serum (FBS) was purchased from Hyclone. 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT), trypsin, streptomycin, penicillin, berberine, and As₂O₃ were obtained from Sigma Co. (St. Louis, MO, USA). 2',7'-Dichlorofluorescein diacetate (DCFH-DA), 4',6-diamidino-2-phenylindole (DAPI), Rhodamine123 were purchased from Molecular Probes (Eugene, OR, USA). Antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Peroxidase-linked secondary antibody was bought from Amersham Life Science (Piscataway, NJ). All other chemicals used were of the highest grade available commercially.

Cell culture

HNB SH-SY5Y cells (American Type Culture Collection, USA) were maintained in MEM and F12 supplemented with 10% (v/v) heat-inactivated FBS and 100 µg/ml penicillin/streptomycin. Cells were maintained in an incubator with a humidified atmosphere of 95% air and 5% CO₂ at 37°C. Experiments were carried out 48 h after cells were seeded onto plates or dishes.

Measurement of cell viability

Cell viability was quantified by MTT assay. Briefly, cells were plated in 48-well culture plates at the density of 5 × 10⁴ cells/well and allowed to adhere at 37°C for 24 h. The following day, various doses of berberine or As₂O₃ were added and the cells incubated for 48 h, after which cell growth and viability were measured using MTT. The absorbance was then measured at 595 nm using a Digiscan Microplate Reader (Assys Hitech, Kornenbourg, Austria). Wells without cells were used as blanks and were subtracted as background from each sample. Results were expressed as a percentage of control.

Nuclear morphology

To assess apoptosis, the nuclei of SH-SY5Y cells were stained with DAPI. Cells were fixed in phosphate buffered saline

(PBS) containing 3.7% paraformaldehyde for 15 min. After fixation, cells were washed twice with PBS and then treated with DAPI in PBS. After three washes, cells were observed under fluorescence microscope.

Analysis of DNA fragmentation by agarose gel electrophoresis

To prepare genomic DNA, SH-SY5Y cells (4 × 10⁶ cells) were incubated with berberine and As₂O₃, or in combination for 48 h, then the cells were detached from 6 cm culture dishes and centrifuged at 100 g for 10 min. The cell pellet was then washed twice with ice-cold PBS and genomic DNA was isolated using a DNA isolation kit (Promega, Basel, Switzerland) according to the manufacturer's instructions. The DNA samples were analysed on a 1.5% agarose gel containing ethidium bromide (1 µg/mL) in Tris-borate-EDTA (TBE) buffer and run for 90 min at 70 V. After electrophoresis, the DNA was visualized under ultraviolet (UV) light and photographed.

Determination of mitochondrial membrane potential

MMP was quantified using the ratio metric probe Rhodamine123. Briefly, cells in 6 cm tissue culture plates were treated with berberine or As₂O₃ alone or combined treatment for the indicated times. Twenty minutes before the cells were harvest, Rhodamine123 was added directly to the culture medium to final concentration of 30 nM. The cells were harvested by trypsinization, washed with 5 ml PBS at 37°C, pelleted by centrifugation, resuspended in 500 µL fluorescence-activated cell sorting (FACS) buffer, and analyzed immediately for Rhodamine123 fluorescence intensity by flow cytometry (FACSCalibur, BD Bioscience, San Jose, CA).

Western blot analysis

Cytosolic protein extracts were prepared as described by Kumagai et al.¹⁵ Briefly, cells were collected by centrifugation at 300 g for 5 min at 4°C and washed with ice-cold PBS. The cell pellet was then resuspended in 500 µL of lysis buffer (20 mM Hepes- KOH, pH 7.5, 210 mM sucrose, 70 mM mannitol; 1.5 mM MgCl₂, 10 mM KCl, 10 µg/ml leupeptin, and 10 µM digitonin). After 10 minutes of incubation at 25°C, the sample was spun at 14,000 g for 15 min, and the supernatant containing cytosolic proteins were stored at -70°C until analysis by 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein extracts were subjected to standard SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Millipore), and probed with appropriate antibodies as described individually in the figure legends. The bound primary antibody was detected using appropriate horseradish peroxidase-conjugated

secondary antibody, and protein was visualized using an enhanced chemiluminescence (ECL) detection kit. β -actin was used as an internal control to confirm that the amounts of protein load were equal.

Measurement of intracellular reactive oxygen species

To monitor the intracellular ROS, we utilized cell-permeable probes, DCFH-DA. Nonfluorescent DCFH-DA, hydrolyzed to DCFH inside of cells, yields highly fluorescent DCF in the presence of intracellular hydrogen peroxide and related peroxides. Therefore, the DCF fluorescence intensity is proportional to the amount of hydrogen peroxide formed intracellularly. At the end of treatment, cells were washed once with PBS, and then incubated in growth media containing $10 \mu\text{M}$ DCF-DA for 30 min at 37°C . Cells were washed once with PBS and suspended in cold PBS, and the fluorescence was monitored by flow cytometry. As a positive control, cells were separately treated with berberine, As_2O_3 alone or combined and processed for ROS detection.

Measurement of lipid peroxidation

Lipid peroxidation assay was based on that described by Ghafourifar et al²¹⁾, with some modifications. Briefly, $50 \mu\text{L}$ of PBS resuspension of harvested cells (1.5×10^6 cells) was added to $50 \mu\text{L}$ of 3.2% SDS in PBS, and incubated for 10 min at room temperature. Following incubation, $150 \mu\text{L}$ of 20% acetic acid (pH 3.5) and $150 \mu\text{L}$ of 0.8% thiobarbituric acid (TBA) in 0.05 N NaOH were added and the mixture was then boiled for 1 h. The mixtures were cooled and extracted with 1 ml each of an n-butanol-pyridine mixture (1:3, v/v) to avoid turbidity. The upper layer (approximately $240 \mu\text{L}$) of each sample was aspirated, and the absorbance was measured at 532 nm. Concentrations of 2-TBA were determined using the extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$. The results were expressed as mol of malondialdehyde (MDA) per mg-protein.

Statistical analysis

The data given in this experiment were expressed as means \pm standard deviation (S.D.). Statistical comparison was carried out with three or more groups using one-way analysis of variance (ANOVA). A difference was considered to be significant at (p -value) < 0.05 .

RESULTS

Combined effects of As_2O_3 and berberine on cell viability

To investigate the combined anticancer effects of As_2O_3 and berberine, we first examined the cytotoxic effects of berberine in SH-SY5Y cells. After SH-SY5Y cells were treated

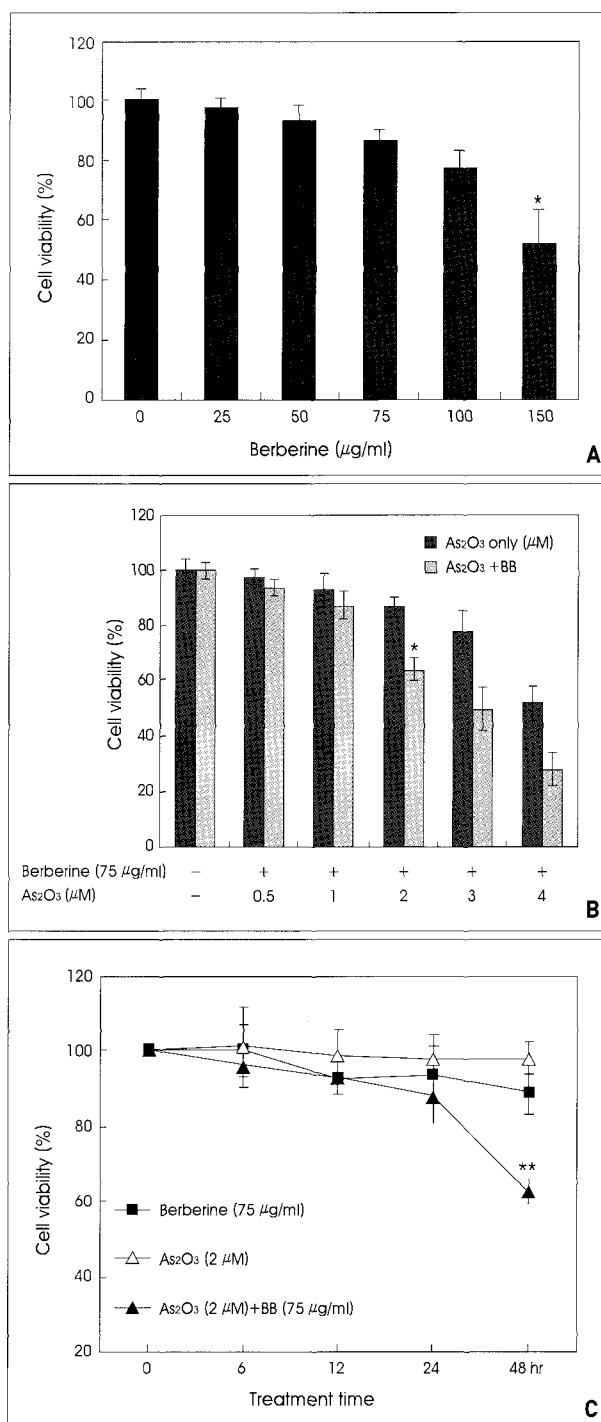


Fig. 1. A : Cytotoxic effect of berberine on SH-SY5Y cells. Cells were treated with various concentrations of berberine for 48 h. Cell viability was determined by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) assay. Results represent the mean \pm S.D. of three independent experiments ($*p < 0.05$). B : Combined treatment of berberine with As_2O_3 decreased the viability of SH-SY5Y cells in a dose-dependent manner. Cells were treated with various concentrations of As_2O_3 and berberine for 48 h. Then, cell viability was determined by MTT assay ($*p < 0.05$). C : Combined treatment of berberine with As_2O_3 decreased the viability of SH-SY5Y cells in a time-dependent manner. Cells were treated with berberine and As_2O_3 for the indicated periods after then, cell viability was determined by MTT assay.

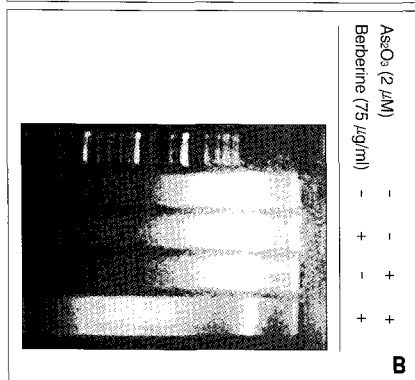
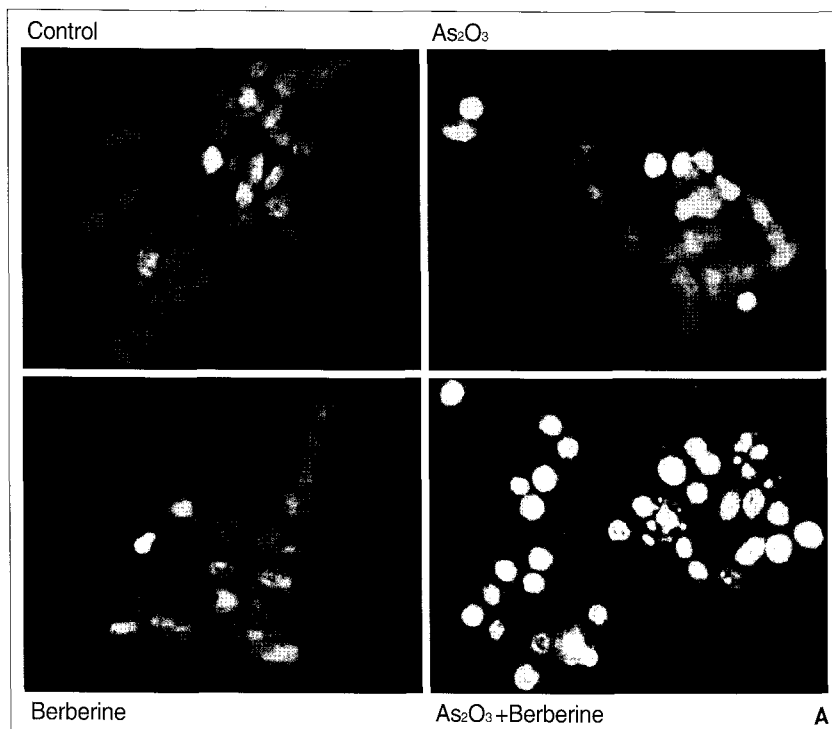


Fig. 2. Combined treatment of berberine with As₂O₃. **A** : Combination treatment with As₂O₃ and berberine induced the chromatin condensation and nuclear fragment of SH-SY5Y cells. Cells were treated with berberine (75 μg/ml) and As₂O₃ (2 μM) and stained with 4',6-diamidine-2-phenylindole (DAPI). The nuclei of cells were observed under fluorescent microscope. **B** : Combination treatment with As₂O₃ and berberine increased the fragmentation of genomic DNA in SH-SY5Y cells. Cell were treated with berberine (75 μg/ml) and As₂O₃ (2 μM) for 48 h. Genomic DNA was extracted, separated on 1.5% agarose gel with size marker, and stained with ethidium bromide to visualized under ultraviolet light.

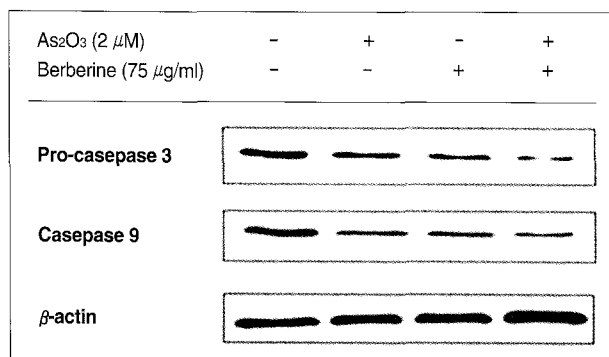


Fig. 3. Combination treatment with berberine and As₂O₃ resulted in a decreased expression of pro-caspase 3 and caspase 9 protein in SH-SY5Y cells. Cells were treated with 75 μg/ml berberine and 2 μM of As₂O₃ for 48 h. The equal amount of protein from cell lysate was subjected on 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto polyvinylidene difluoride-nitrocellulose filters (PVDF) membranes, and immunoblotted with anti-procaspase 3, anti-caspase 9 and anti-β-actin antibodies, respectively. The immunoreactive signals were visualized by enhanced chemiluminescence (ECL) kit.

with various doses of berberine for 48 h, cell viability was measured by MTT assay (Fig. 1A). These experiments for berberine were done to identify a concentration that produced modest growth inhibition to test subsequently its combination with As₂O₃. We noticed that a dose of berberine as low as 75 μg/ml caused little effects on cell viability. However, berberine significantly enhanced cytotoxicity (61%), when combined with 2 μM As₂O₃. Hence, 75 μg/ml berberine and 2 μM As₂O₃ were selected as the dose used for the combination effects in SH-SY5Y cells.

The viability of SH-SY5Y cells treated with berberine and As₂O₃ was inversely proportional to the dose of As₂O₃. In the concentration of As₂O₃ more than 2 μM showed prominent synergistic effect (Fig. 1B). Also, the combined treatment of two drugs showed a significant cytotoxicity in a time-dependent manner (Fig. 1C).

Apoptosis induced by combined treatment of As₂O₃ and berberine

In order to define the mode of cell death induced by combined treatment of As₂O₃ with berberine, we examined nuclear morphology and DNA fragmentation. After SH-SY5Y cells were treated with 2 μM As₂O₃ and 75 μg/ml

berberine, nuclear morphology was examined by DAPI staining. In contrast to control group and single treated groups (berberine or As₂O₃), combined treatment group of As₂O₃ with berberine showed chromatin condensation, DNA fragmentation under fluorescent microscopy (Fig. 2A). To reconfirm the DNA fragmentation, genomic DNA was extracted and electrophoresed on 1.5% agarose gel. Combined treatment with As₂O₃ and berberine induced apoptic DNA fragmentation, however, single treated groups with 75 μg/ml berberine or 2 μM As₂O₃ were not (Fig. 2B).

To address the particular role of caspase in berberine and As₂O₃ induced apoptosis, we investigated activities of caspase-3, -9. The western blot analysis of pro-caspase-3 and caspase-9 protein is illustrated in Fig. 3. As presented, combined treatment of berberine and As₂O₃ decreased expression of pro-caspase-3 and caspase-9 protein in SH-SY5Y cells markedly.

Combined treatment with As_2O_3 and berberine-induced apoptosis is associated with a reduction of mitochondrial membrane potential and alteration of Bcl-2 family proteins

A decline of the MMP may be an early event in the process of cell death. The loss of the MMP is the marker for the activation of mitochondrial pathway, which is regulated by pro- and anti-apoptotic proteins of the Bcl-2 family. Since disruption of the MMP is a critical step in cells undergoing apoptosis, we evaluated whether combined treatment with As_2O_3 and berberine had any effect on the MMP by using Rhodamine123 as a marker of mitochondrial membrane integrity. Disruption of MMP causes a decrease in FL-1 fluorescence as analyzed by flow cytometry. As indicated in Fig. 4, there was no substantial change of the MMP after treatment with 2 μM As_2O_3 or 75 $\mu g/ml$ berberine alone. However, when the cells were treated with As_2O_3 and berberine in combination, a markedly decreased Rhodamine123 fluorescence was observed, indicating disruption of the MMP in these cells.

It has been recognized that the proteins of Bcl-2 family play crucial roles in regulation of apoptosis by functioning as inhibitors (Bcl-2, Bid or Bcl-x/L) of cell death. We therefore reasoned to determine the effect of berberine on the expression of these proteins. The results revealed that the combined treatment with As_2O_3 and berberine resulted in a marked reduction in the levels of protein Bcl-2, Bid and Bcl-x/L (Fig. 5).

Combined treatment with As_2O_3 and berberine in significantly increased intracellular ROS generation and lipid peroxidation

Because of the mitochondrial respiratory chain on the inner mitochondrial membrane is a major intracellular source of ROS, we next examined whether combined treatment with As_2O_3 and berberine induces ROS generation. We examined the level of ROS production in combination with berberine and As_2O_3 using DCF-DA, ROS-sensitive probe. In contrast to the control and As_2O_3 or berberine alone, treatment with 2 μM As_2O_3 and 75 $\mu g/ml$ berberine markedly induced intense fluorescence in SH-SY5Y cells (Fig. 6). We next addressed the effect of combined treatment with As_2O_3 and berberine on the content of intracellular lipid peroxidation products (thiobarbituric acid reactive substance). Although cells treated with As_2O_3 or berberine alone showed no

significant differences in lipid peroxidation, when cells were combined with As_2O_3 and berberine for 48 h, the rate of lipid peroxidation significantly increased in 2 folds (Fig. 7).

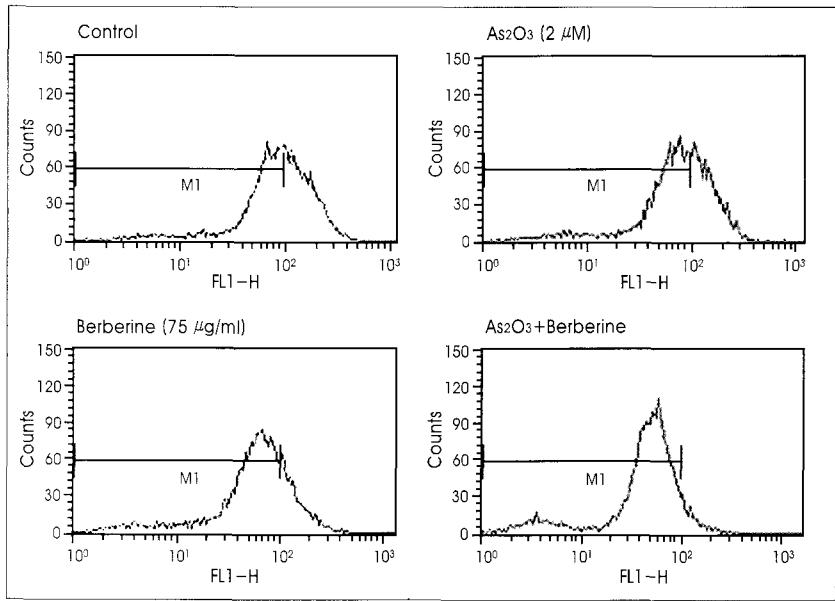


Fig. 4. Combined treatment of berberine and As_2O_3 induced the mitochondrial membrane potential transition (MMPT) in SH-SY5Y cells. Cells were treated with 75 $\mu g/ml$ berberine and 2 μM As_2O_3 48 h. Then, cells were incubated with 50 nM Rhodamine123 for 30 min at 37°C and used to analyze MMPT with flow cytometry. The horizontal axis indicates the relative fluorescence intensity and the vertical axis denotes the relative cell number.

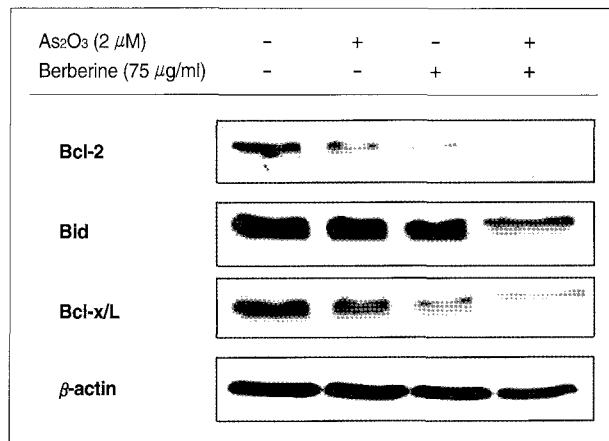


Fig. 5. Combined treatment of berberine and As_2O_3 decreased the expression of anti-apoptotic Bcl-2 proteins in SH-SY5Y cells. Cells were treated with 75 $\mu g/ml$ berberine and 2 μM of As_2O_3 for 48 h. The equal amount of protein from cell lysate was subjected on 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred onto polyvinylidene difluoride-nitrocellulose filters (PVDF) membranes, and immunoblotted with anti-Bcl-2, anti-Bid, anti-Bcl-x/L, and anti- β -actin antibodies, respectively. The immunoreactive signals were visualized by enhanced chemiluminescence (ECL) kit.

significantly differences in lipid peroxidation, when cells were combined with As_2O_3 and berberine for 48 h, the rate of lipid peroxidation significantly increased in 2 folds (Fig. 7).

DISCUSSION

Berberine has been used to treat diabetes for more than 1,000 years in the long history of chinese medicine. Berberine

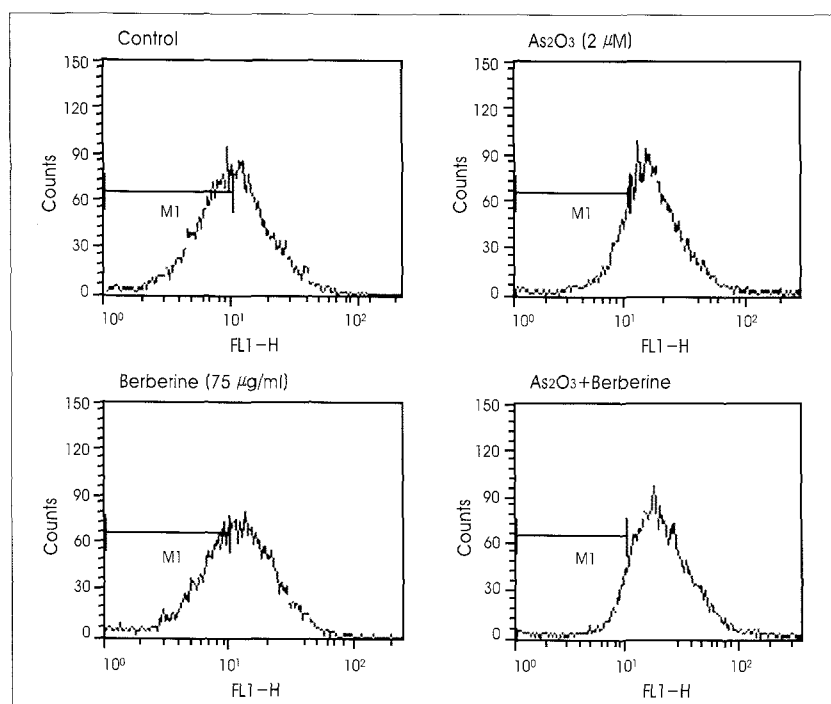


Fig. 6. Combination treatment with berberine and As₂O₃ increased the intracellular reactive oxygen species (ROS) in SH-SY5Y cells. Cells were treated with 75 μg/ml berberine and 2 μM As₂O₃ for 32 h. After harvesting, cells were stained with 2',7'-dichlorofluorescein diacetate (DCFH-DA) dye and subjected on flow cytometric analysis to estimate intracellular ROS levels.

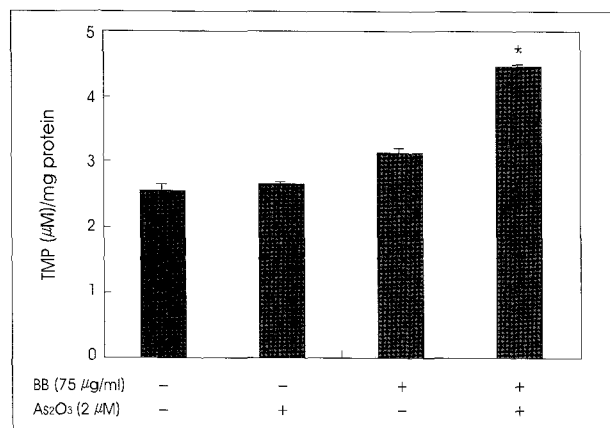


Fig. 7. Combination treatment with berberine and As₂O₃ increased the lipid peroxidation of SH-SY5Y cells. Cells were treated with berberine (75 μg/ml) and As₂O₃ (2 μM) for 48 h. Cell homogenates were mixed with thiobarbituric acid (TBA) in glass test tubes and incubated in a boiling water bath for 60 min. The level of lipid peroxidation was expressed in absorbance at 532 nm using tocopherol-mediated peroxidation (TMP) as an external standard, and expressed as mean ± S.D. (n=3) (*p<0.05).

has a wide range of pharmacologic actions such as antidiarrhetic, antimicrobial, anticancer, anti-inflammatory, and antiarrhythmic activities^{7,26}. Berberine was also shown to inhibit DNA and protein synthesis, cell cycle progress of arrest, and the *in vitro* growth of a number of human cancer cell lines, however, the molecular mechanisms underlying berberine-induced apoptosis are not yet well defined⁷.

Although As₂O₃ has potent activity against cell growth

in a series of leukemia cell lines, little information is available regarding this compound's effect on cell growth in solid tumor cell lines⁴. The mechanisms of As₂O₃ cytotoxicity are not known completely, but preclinical studies have provided insight into the processes involved. The mechanisms include cellular differentiation, induction of apoptosis, and inhibition of angiogenesis⁴. As₂O₃-induced apoptosis has been described in three major apoptotic mechanisms; mitogen-activated protein (MAP) kinase, activation of caspase pathway, and production of ROS^{2-4,14,24}.

Because As₂O₃ at higher concentrations induces many side effects (ventricular arrhythmia, skin reaction, peripheral neuropathy, electrolyte changes, leukocytosis, hepatic dysfunction, GI reactions, etc.) as well as association linking carcinogenesis with chronic arsenic exposure, low-dose combination therapy is required^{12,9,10,17,27,28}. In our study, low

dose combination of two chemotherapeutic agents (75 μg/ml berberine + 2 μM As₂O₃) decreased cell viability markedly, however each of low dose single agent (75 μg/ml berberine or 2 μM As₂O₃) could not decreased cell viability significantly. Combination of berberine and As₂O₃ at non-toxic level has synergistic effects of apoptosis on HNB SH-SY5Y cells *in vitro*, therefore, we can expect that clinically it may reduce the side effect of therapeutic agents^{8,23}.

To determine apoptotic effects of combination chemotherapy (As₂O₃+berberine) in HNB SH-SY5Y cell line, we had investigated the cell morphology, ROS production, lipid peroxidation, mitochondrial membrane potential transition (MMP), and expression of apoptosis induced protein. The treatment of SH-SY5Y cells with combination of berberine and As₂O₃ was sufficient to induce apoptosis and cleavage of several key proteins related to the mitochondrial death pathway, which was examined by western blot analysis^{6,7,13}. These experiments demonstrated that the combined treatment of As₂O₃ and berberine significantly activates the mitochondrial apoptosis pathway which are regulated by the anti-apoptotic proteins of the Bcl-2 family^{8,23,25}. In mitochondria-mediated pathway, cytochrome c and other apoptogenic proteins are released from the mitochondrial intermembrane space into the cytosol. Once released, cytochrome c binds to Apaf-1 and induces sequential activation of caspase-9 and caspase-3. Activation of caspase-8, result from death factors (e.g. Fas

ligand, tumor necrosis factor- α) bind to their relevant cell-surface receptors, provides a link between receptor-mediated and mitochondria-mediated pathways of apoptosis. Caspase-8 activation induces cleavage of Bid to truncated Bid (tBid) that triggers Bax activation, resulting in change in mitochondrial permeability and thereby release of cytochrome c into the cytosol. Hwang et al.⁷⁾ reported that cytotoxicity of berberine is due to the induction of apoptosis which activates procaspase and DNA fragmentation. Furthermore, the activation of caspases results to decrease the bcl-2, Bid, and Bcl-x/L. Therefore, the berberine-induced apoptosis could be almost completely inhibited by caspase inhibitors.

One of mechanisms of As₂O₃-induced apoptosis is through activation of caspases. As₂O₃ activates these proteases, which play an important role in the degradation phase of apoptosis, in NB4 cell lines⁴⁾. A key event in the effector phase of apoptosis is the progressive permeabilization of the mitochondrial membrane secondary to the action of permeability transition pore complex. As₂O₃ can lead to changes of MMP and increased membrane permeability as a result of degradation phase of apoptosis⁴⁾. The results of our study were also consistent with the findings of aforementioned. The combination treatment of As₂O₃ with berberine decreased expression of pro-caspase 3, caspase 9 protein, bcl-2, Bid, Bcl-x/L, and induced the MMPT in HNB SH-SY5Y cells.

ROS are free radicals that react with all biological macromolecules, modify the structure and function of proteins as well as cause oxidative damage to DNA^{12,14)}. Hydrogen peroxide (H₂O₂) is a common intermediate in the oxidative metabolic process, which has been shown to induce DNA damage in human cells^{4,5,27,28)}. Despite varied antioxidant defenses occurring in cells, there is always a steady state concentration of ROS presents^{1,3,4)}. ROS damages cellular DNA through oxidative stress-induced destruction of pyrimidine and purine bases and single strand breaks. A critical component of the cellular response to oxidative stress is the glutathione redox system, a known modulator of arsenical induced cell killing^{1,4,20)}. As previously studied by other authors^{4,11)}, our study revealed that combined treatment with As₂O₃ and berberine increased the intracellular ROS than single treatment in HNB SH-SY5Y cells.

CONCLUSION

Combined treatment of As₂O₃ and berberine at non-toxic dose level induced activation of apoptotic signal transmission system, DNA fragmentation and caspase family protein, decreased expression of MMPT, and increased production of intracellular ROS in SH-SY5Y cells. These results suggest that the possibility of the combined treatment of two

chemotherapeutic agents with low concentration can improve cytotoxic effect for cancer cells with minimal side effects.

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