Open

Citation: J Med Discov (2020); 5(4):jmd20055; DOI:10.24262/jmd.5.4.20055

Research Article

DMSO inhibits growth and induces apoptosis through extrinsic pathway in human cancer cells

Tang Hu[1,*], Ariana Villarroel[1], Angela Duff[1], Izabella Ruiz[2], Adrian Almeida[1]

[1]Department of Biology, College of Arts & Sciences[1], BSN Program, College of Nursing and Health Sciences[2], Barry University, Miami Shores, Florida 33161.

Abstract Dimethyl sulfoxide (DMSO) is a small molecule that is widely used as a solvent, cryoprotectant of cells, and pharmaceutical agent for many years. Despite its widely applications, both cytotoxic and stimulatory effects of DMSO have been reported in recent years. To have a general concept on DMSO, in this study, we investigated the effect of DMSO on both suspension and solid cancer cells and its action mechanisms. We found that DMSO at relatively low concentrations significantly inhibited proliferation and caused death in both types of cells. Cytology examination showed that the cells treated with DMSO displayed marked cellular damages: cytoplasm shrank or broken; the nuclei appeared as fragments or dots; and the cell bodies decreased in size. The results from Western blotting demonstrated that DMSO downregulated the expression of CDK2 and cyclin A. DNA analysis exposed that the cells in the presence of DMSO exhibited clear nuclear DNA ladders. In consistent with the DNA fragmentation, the apoptotic proteins, caspase 3, but not caspase 9, was activated in the cells treated with DMSO. In summary, our study demonstrated that DMSO has a significant effect on the cellular process in both suspension and epithelial-derived cancer cells, which is liked to cell cycle regulatory and apoptotic molecules. Our data provides supplementary information on the mechanisms responsible for the DMSO-induced cytotoxic effect.

Keywords: Dimethyl sulfoxide, apoptosis, cell growth, cytotoxicity

How to cite: Tang Hu et al.,DMSO inhibits growth and induces apoptosis through extrinsic pathway in human cancer cells. J Med Discov (2020); 5(4):jmd20055; DOI:10.24262/jmd.5.4.20055; Received July 25th, 2020, Revised September 08th, 2020, Accepted September 15th 2020, Published October 05th, 2020.

Introduction

Dimethyl sulfoxide (DMSO) is a small organic molecule composed of one hydrophilic sulfoxide and two hydrophobic methyl groups with the formula $(CH_3)_2SO$. This unique structure makes DMSO soluble in both water and organic media. Moreover, soluble DMSO can penetrate rapidly through the biological membranes (1). Although DMSO has long been reported to have some kind of toxicity on cells (2-7), it is assumed that cytotoxic effects of DMSO are negligible (6-8), especially when DMSO concentration is below 10% (v/v). Since 1960's, the application of DMSO has been involved in many areas. For laboratory research, DMSO is broadly used as an efficient solvent for many chemicals with poor solubility in water, as an enhancer for drug delivery (9-10) and as a common cryoprotectant (5-10%) for preventing cellular damage during freeze-thaw process (11-12). Clinical studies show that DMSO can reduce pain symptoms caused by different diseases such as inflammation, osteoarthritis, and rheumatoid arthritis (13-15). Intravenously, DMSO stopped hematuria symptoms in patients with bladder amyloidosis (16). Alzheimer's is a disease with no known cure. The disease is characterized by intracellular aggregates of hyperphosphorylated tau protein. One paper published in *PloS One* reported that DMSO can inhibit the phosphorylation of Tau protein in animal model (17),

* Correspondence:Tang Hu, Ph.D., Department of Biology, College of Arts & Sciences, Barry University, Miami Shores, Florida 33161, USA. E-mail: xthu@barry.edu.

raising a hope that DMSO may help to treat Alzheimer's disease. However, significant toxicities and stimulatory effects of DMSO on some types of animal cells have been reported in recent years. In mice, intraperitoneal administration of DMSO led to severe loss of lymphocytes and reduction in the spleen weight (18). In goats, high concentrations of DMSO decreased viability of the skin fibroblasts, whereas the low concentrations of DMSO stimulated cell proliferation (19). Both stimulatory and inhibitory/cytotoxic effects of DMSO on human endothelial cells and lymphocytes have also been observed (20-22).

Despite recent reports of the cytotoxic effect of DMSO on a variety of cells, the mechanisms by which DMSO mediates its inhibitory or cytotoxic effect are largely unknown. It is also unclear whether suspension leukemia and solid carcinoma cells have the same response to DMSO. Thus, further research is needed to assess whether the cytotoxic effect of DMSO is negligible in its applications in more types of cells and whether the cytotoxic effect finally results in necrosis, apoptosis or inhibition of mitosis. To obtain a general conclusion, in the present study, we investigated the inhibitory effect of DMSO on the proliferation of both suspension myeloid leukemia and epithelial-derived adhesion cancer cells as well as the possible mechanisms responsible for the inhibitory effect.

MATERIALS AND METHODS

Maintenance of Cell Lines. Human TF-1a, MV4-11 (leukemia cells), Hep-G2 (liver cancer cells) and MCF7 (breast cancer cells) cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). TF-1a and MV4-11 cells were routinely maintained in Iscove's Modified Dulbecco's Media (IMDM) supplemented with 10% fetal bovine serum (FBS), 1% of Streptomycin-Penicillin-Glutamine (SPG); Hep-G2 and MCF7 cells were cultured in Eagle's Minimum Essential Medium (EMEM) with 15% FBS and 1% SPG. All the cells were cultured at 37 °C in humidified air containing 5% CO2. The culture media and FBS were purchased from Life Technology (Grand Island, NY) and ATCC (Manassas, VA), respectively.

Reagents and Antibodies.DMSO and trypan blue were obtained from Sigma ((St. Louis, MO). XTT assay kit was purchased from ATCC (Manassas, VA). RIPA buffer, Wester Blotting chemiluminescence luminol reagent, antibodies against CDK2, cyclin A, and cyclin E were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The antibodies against GAPDH, total caspase 3, activated caspase 3 and caspase 9 were purchased from Santa Cruz Biotechnology and Cell Signaling Technology (Danvers, MA), respectively. The quick apoptotic DNA ladder Detection Kit was purchased from BioVision (Milpitas, CA)

Assay of cell proliferation. Cell proliferation was measured by indirect colorimetric immunoassay, XTT. Briefly, the cells were grown in microtiter plates in a final volume of 100µl of the culture media in the presence or absence of DMSO. After 12 to 72 h incubation, the XTT labeling reagents (50µl) were added to the cells, and the cells were incubated for 4h at 37 °C in a humidified atmosphere air containing 5% CO_2. After solubilizing, the formazan dyes were quantitated using a microtiter plate

(ELISA) reader at a wavelength of 470 nm, as recommended by the manufacturer (ATCC, Manassas, VA).

Trypan blue viability assay. Cells were diluted in 0.4% Trypan Blue solution in 1:1 ratio and incubated at room temperature for 4 min, after which the cells were loaded into a hemocytometer chamber. The blue and non-blue cells were counted under a light microscope.

Cytology examination of cells. Cells were cultured in the medium at the condition described under "Maintenance of Cell Lines". During log-phase growth, MV4-11 and TF-1a cells treated with or without DMSO were collected, and cytospins were prepared by centrifuging aliquots of harvested cell suspensions at 1000 rpm for 5 min, after which the cells were stained with Giemsa. Hep-G2 and MCF7 cells were cultured on the culture chamber slides for 48h, after which the cells were fixed and stained with Giemsa. The cell morphology and nuclear fragmentation (cytological evidence of apoptosis) were determined by examining morphological alterations in both the cytoplasm and nucleus under a light microscope at 1000X.

Detection of DNA Fragmentation. DNA fragmentation was detected using the Quick Apoptotic DNA ladder detection kit with minor modifications. Briefly, the cells treated without or with DMSO were pelleted in a 1.5 ml microcentrifuge tube, after which the cells were washed with 1 x PBS and lysed with TE lysis buffer for 30 min on ice followed by centrifugation in an Eppendorf microcentrifuge for 10 min at 4^{o}C. After enzyme A and B treatment, the DNA was precipitated in the presence of ammonium acetate and isopropanol solutions. The DNA pellets were then dissolved in DNA suspension buffer. A quote of DNA was loaded into 1.2% agarose gel for running until the yellow dye (the suspension buffer) reached to the edge of the gel. After the electrophoresis was completed, the gel was removed from the electrophoresis chamber and placed on a piece of plastic wrap on a flat surface. Then, a piece of ethidium bromide card was placed on the top side of the gel for 15 min. The ethidium bromide-stained DNA was visualized under UV light.

Preparation of whole cell lysates. Cell pellets were harvested by centrifugation and lysed in 1x RIPA buffer (Santa Cruz, Biotechnology, Dallas, TX) supplemented with PMSF, sodium orthovanadate and protease inhibitor cocktail, and incubated on ice for 30 min. Subsequently, the lysates were sonicated and centrifuged at 14,000g at 4^{o}C for 15 min. The supernatants were carefully transferred to a fresh microfuge tube and amounts of total proteins were measured with a spectrophotometer (BIO-RAD), after which the lysates were used for Western blotting analysis.

Western Blotting Assays. Lysates containing equal amounts of proteins were separated on a 10 –15% SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. The proteins in the membrane were then immunoblotted with an appropriate antibody for 1 h at room temperature or overnight at 4°C. The first antibody containing solution was then removed and the blot was washed three times in 1x TBST buffer [20 mM Tris, 137 mM NaCl (pH 7.6), and 0.1% Tween 20]. Subsequently, the blot was incubated with an HRP-conjugated secondary antibody at room temperature for 1 h, and the expression of proteins was detected by chemiluminescence (Santa Cruz, Biotechnology, Dallas, TX), followed the instruction provided by the manufacturer.

Immunoprecipitation. Cells were lysed in RIPA lysis buffer and immunoprecipitation was performed using cell lysates containing 400μg of total proteins. The lysates were incubated with an appropriate antibody for 2 h or overnight with agitation at 4 °C. Subsequently, 30μl of protein A-Sepharose or protein G-agarose beads were added to the lysates and the incubation continued for another 1 h at 4 °C. Immune complexes were washed three times with 1x lysis buffer and then resuspended in 2x SDS sample buffer. Lysates in the sample buffer were subjected to Western blotting analysis and detected by the chemiluminescence reaction as described in the section of "Western blot analysis" above.

Statistical analysis. All results were expressed as mean ± SD of data obtained from three or more independent experiments. The Microsoft Excel program was used for all statistical analysis.

RESULTS

DMSO inhibited the growth of human myeloid leukemia and epithelial cancerous cells.

In recent years, XTT assay has been a common and convenient method to measure cell proliferation and cell viability. To study the possible effect of DMSO on cell proliferation, DMSO was added to myeloid leukemia TF-1a, MV4-11, and epithelial cancerous Hep-G2 and MCF7 cells in log-phase growth. After 12-72 h the cells were harvested, and the cell proliferation was assessed with XTT reagent. The addition of DMSO, variably, but significantly, inhibited the growth of MV4-11, TF-1a, Hep-G2, and MCF7 cells. The inhibitory effect of DMSO was dose-dependent, with initial inhibitions being observed at the concentrations of 2% in the myeloid leukemia cells. The significant inhibition of the cell growth was observed when 4% or higher concentrations of DMSO were applied to the cell culture, with a maximal inhibitory effect being observed at 10% DMSO at which the inhibitions of MV4-11, TF-1a and Hep-G2 cells are 58%, 55%, and 69%, respectively (Fig. 1A). The DMSO-induced inhibition is also time-dependent with the earliest inhibition being observed at 24 h and reached to a maximum at 72 h after initiated DMSO treatment. The growth inhibition of MV4-11 and TF-1a cells in response to 5% DMSO for 24 h was 29% and 30%, respectively. When the cells were treated with DMSO for 72 h, the inhibition of the MV4-11 and TF-1a cells was increased to 70% and 72%, respectively (Fig. 1B).

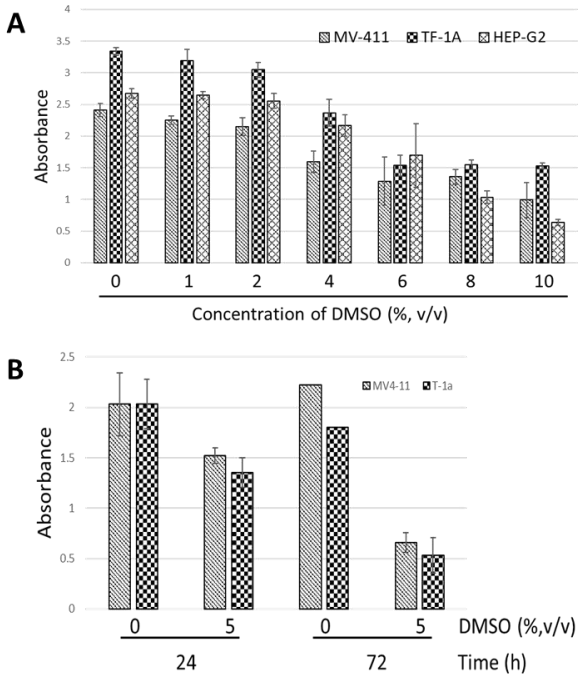

Fig. 1 DMSO inhibits growth of human myeloid leukemia and epithelial cancerous cells. The cells were grown in microtiter plates in a final volume of 100μl IMDM in the presence or absence of DMSO. After 48h (A) or 24-72h (B) incubation, the XTT labeling reagents (50μl) were added to the cells, and the cells were incubated for 4h at37°C in a humidified air containing5% CO2, followed by adding 100μl of the Solubilization solution into each well for overnight. After solubilizing, the formazan dyes were quantitated using a microtiter plate (ELISA) reader at a wavelength of 550nM in a spectrophotometer.

DMSO induced cell death.

To study whether the inhibition of cell growth was caused by cell death, trypan blue stain assay was applied to examine cell viability. This method is based on the principle that live cells possess intact cell membranes that exclude the blue dyes, whereas dead cells or the cells with damaged membranes lose this ability. The outcome from the trypan blue stain showed that control MV4-11 and TF-1a cells (without DMSO) had cell death rates being 3% and 2%, respectively, after 48h incubation in the complete IMDM media. Addition of DMSO at a concentration of 5% caused significant cell killing with the death rates being increased to 15 % and 19% in MV4-11 and TF-1a cells, respectively. However, further increase of DMSO concentrations caused little more cell death in both leukemia cell lines (Table 1). A similar cell killing pattern was also observed in epithelia HepG2 and MCF-7 cells (data not shown). Under inverted microscope, the intensity of MV4-11, TF-1a, and Hep-G2 in culture dishes are markedly reduced in the cells treated with DMSO, which is clearly dose-dependent: the more DMSO, the less intensity of cells being observed. In contrast, the cell debris observed in the cells treated with DMSO is proportionally increased following the increase of DMSO concentrations. Most Hep-G2 cells in the absence of DMSO appear as a monolayer adhering to the culture substrate after 48h incubation in the complete culture medium. Addition of DMSO disrupted the formation of the monolayer and some Hep-G2 cells detached from the substrates and formed clusters in different sizes that is floating in the culture liquid (Fig. 2a). Next, we carried out cell staining to analyze the morphology of the cells and their intracellular structures in the absence and presence of DMSO. The MV4-11, TF-1a, Hep-G2, and MCF7 cells treated with or without DMSO for 48-72h were collected and stained with Giemsa. Then, the cell morphology was examined under a light microscope at 1000X amplifications (Fig. 2B). MV4-11 and TF-1a cells show relatively a homogeneous population of medium-size cells with the appearance of blasts. They have a smooth cytoplasmic border with big nucleus/cytoplasm ratio. MV4-11 cells are about 1/3 to 1/4 smaller than TF-1a cells in size (in diameter). Application of DMSO decreased the cell numbers and caused cell morphology changes indicated by decreased cell density, shrinkage of cell body, and the formation of cell blebbing. DMSO also caused breakage of cell nuclei, evidenced by appearance of multiple nuclear fragments/dots in nondividing cells. The amounts of nuclear dots/fragments are dose-dependent, with initial nuclear fragments and dots being observed in all four types of the cell lines treated with 2% DMSO for 48 hours. DMSO at concentrations 4% or higher caused much more nuclear debris, dots, and fragments. Both Hep-G2 and MCF7 cells show epithelial-like morphology in culture. Without staining, MCF7 cells have very weak contrast in culture, making their morphology less clear. After staining, MCF7 cells are clearly visible with multiple nuclei to be found in many cells. Both Hep-G2 and MCF7 cells show cytology changes in response to DMSO, which are similar to the changes in the leukemia cells tested. In summary, all these 4 types of the cells treated with DMSO show three common characteristics changes in response to DMSO: (1) shrinkage of cell body (2) decrease of cell numbers (3) formation of nuclear debris. In addition, TF-1a, Hep-G2, and MCF7 cells show clear nuclear condensation.

Table 1. Cell viability detected by Trypan blue assay. Cells in log phase were collected and treated with or without DMSO for 72 hours were collected and their viability was determined by Trypan blue exclusion assay (see Materials and Methods). The non-blue and blue cells represent living and dead cells, respectively.

Table 1 Cell death detected by trypan-blue assay

Cell Line	DMSO Concentration (%)	Non-Blue Cells	Blue Cells	Death Rate (%)
MV4-11	0	198.3 ± 14.3	7.0 ± 1.1	3.5
	5	181.7 ± 20	25.3 ± 3.3	13.9
	10	223.0 ± 23.3	38.3 ± 4.0	17.2
TF-1a	0	249.0 ± 25.3	9.7 ± 1.4	3.9
	5	204.7 ± 26.3	38.5 ± 3.4	18.8
	10	202.9 ± 19.7	40.4 ± 5.0	19.9
HepG2	0	202.2 ± 17.3	3.3 ± 0.5	1.6
	5	237 ± 23.0	40.0 ± 3.6	16.9
	10	263.1 ± 29.7	48.3 ± 4.9	18.4

Cells treated with or without DMSO for 72 hours were collected and their viability was determined by Trypan blue exclusion assay (see Materials and Methods). The non-blue and blue cells represent living and dead cells, respectively.

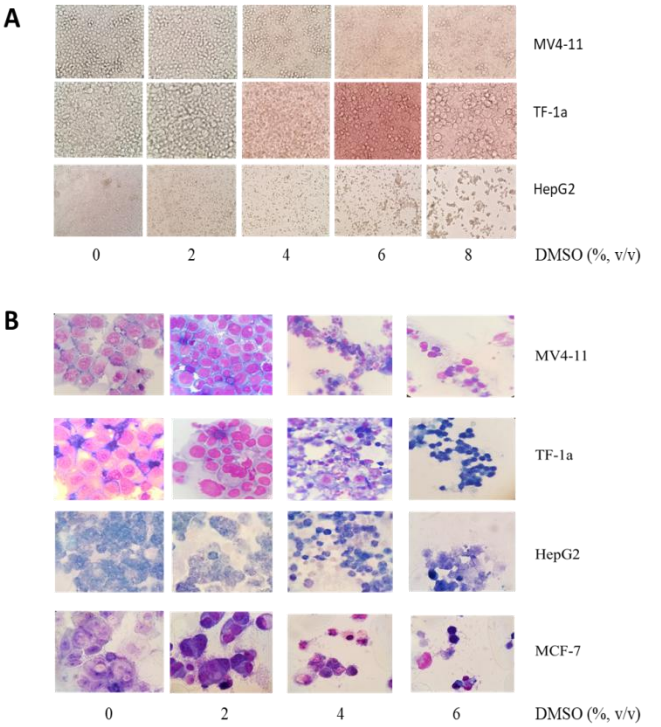

Fig. 2. **DMSO causes alterations of cell morphology**. The cells in log phase were collected and treated with or without DMSO for 48 hours, after which the cells in the culture dishes were observed under an inverted microscope, and the morphologies were photographed at 100X (A). A portion of MV4-11 and TF-1a cells were collected and their slides were prepared by centrifugation in a cytospin. The cells on the slides were stained with Giemsa. Hep-G2 and MCF7 cells on the culture chamber slides for 48h were fixed and stained with Giemsa (B).

Down-regulation of CDK2 and cyclin A was linked to DMSO-induced growth inhibition

Usually cytotoxic effect of a chemical compound usually does not involve in the expression of cell cycle regulatory molecules, whereas the inhibition of cell division or apoptotic suicide of cells is often a result of inhibiting or activating some related proteins. In addition, it has been reported that loss of some cyclins and CDKs is prerequisite of apoptosis (Spyridopoulos I 2001). Thus, next we examined the expression of several cell cycle regulatory molecules with a focus on CDK2 and its regulatory subunits, cyclin A and cyclin E, because CDK2-cyclin complexes play a key role in cell progress from G1 to S phase. Proliferating TF-1a cells not exposed to DMSO expressed CDK2 at 33–35 kDa, and cyclin A at about 50 kDa, Addition of DMSO at 5% caused a considerable decrease in the levels of CDK2 and cyclin A detected at 48h, respectively. The low concentration of DMSO (0.5%) had no significant effect on the expression of CDK2 and cyclin A. Subsequently, we analyzed the expression of CDK2-cyclin A complex in the cells treated with or without DMSO. Similarly, the CDK2-cyclin A complexes were significantly downregulated in the cells treated with DMSO detected by immunoprecipitation. The downregulation of the complexes is clearly dose-dependent because the low concentration of DMSO had no effect on the amount of CDK2-cyclin A complexes (Fig. 3). DMSO had no effect on the expression of cyclin E (data not shown).

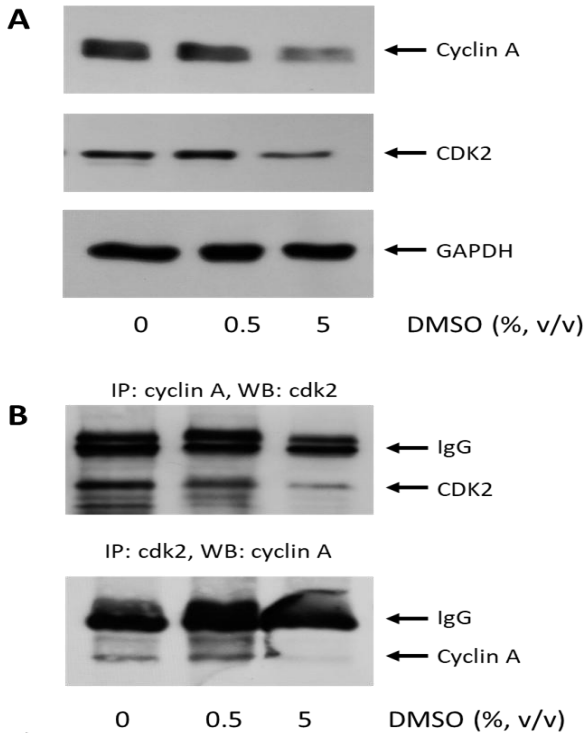

Fig. 3. DMSO inhibits CDK2, cyclin A, and CDK2-cyclin A complex. TF-1a cells treated with or without DMSO for 24 h at 37 °C, 5% CO2 were collected and lysed. Aliquots of the extracts were subjected to Western blot (A) or immunoprecipitation analysis (B).

DMSO caused DNA fragmentation and activated caspase 3 but not caspase 9

The changes in the cell morphology and nuclear debris in DMSO treated cells suggest that DMSO might cause apoptosis in the cells tested. The inhibition of CDK2-cyclin complexes by DMSO provides further support for this possibility. To test this hypothesis, we first performed DNA ladder detection using the Quick Apoptotic DNA ladder detection kit, because DNA fragmentation is a hallmark of apoptosis. As seen from Fig. 4A, control cells show one DNA band without clear fragmentation, whereas the cells treated with DMSO show multiple smear DNA fragments. The increase of DNA fragments is proportional to the concentration of DMSO with maximal numbers of DNA fragmentation being observed at a concentration of 8%. It has been reported that caspase 3 is a key protease that becomes activated during the early stages of apoptosis (23-24) and is required for the formation of DNA fragmentation (25). Activated caspase 3, which is found in cells undergoing apoptosis, consists of multiple subunits, including molecular sizes 19,000, 17,000, and possible other smaller subunits, depending on the degree of caspase 3 activation and cell types (26). These subunits are derived from a molecular size 32,000 proenzyme. To determine whether DMSO induced caspase 3 activation, Western blot analysis was performed with an anti-caspase 3 or -activated caspase 3 antibodies (Fig. 4B). MV4-11 and TF-1A cells in the absence of DMSO expressed 32-kDa proenzyme with barely detectable small protein bands. However, a 24-h incubation with DMSO significantly decreased the expression of the 32-kDa protein and induced appearance of the smaller bands detected by anti-caspase antibody (Fig. 4B, top panel), which is dose-dependent with the maximal changes were detected in the cells treated with 5% DMSO. To determine whether the small proteins are active caspase 3, the same membranes are stripped and the expression of activated caspase 3 was detected with anti-activated caspase 3 antibody. As shown in the middle panel of Fig. 4B (middle panel), the control cells expressed very low levels of 19- and 17-kD activated caspase 3 and addition of DMSO at 5% greatly upregulated the expression of 19- and 17-activated caspases. In addition, an additional smaller protein at about 14 kDa was also detected in the DMSO-treated TF-1a cells. DMSO at 0.5% did not have a significant effect on the activation of caspase 3 as compared with the control cells. To find out if the other caspase pathways were also involved in DMSO-induced

apoptosis, we next examined the expression of caspase 9. Our data showed that DMSO did not significantly affect the activation of caspase-9 in both MV4-11 and TF-1a cells (Fig. 4B bottom panel).

DISCUSSION

For many years, dimethyl sulfoxide (DMSO) has been used as a solvent for water-insoluble substances, a vehicle for drug therapy, and a cryoprotectant at concentrations at ≤ 10% for cultured cells. DMSO is also a prescription medicine and dietary supplement. A good reason behind the ubiquitous and wide applications of DMSO is that DMSO has been generally accepted as nontoxic chemical compound below 10%(v/v) or the cytotoxic effects of DMSO are negligible (6, 7, 8), in many applications. However, the cytotoxic effect of DMSO has gained researchers' attention in recent years and has been observed in a number of cell types including neuron cells (8), red cells (27), lymphocytes (22), leukemia cells (21) and embryos (28). Despite these discoveries, there are still many inconsistent reports regarding whether the cytotoxic effects of DMSO are negligible and whether the cytotoxic is cell type dependent. Moreover, the nature of the cytotoxic effect of DMSO is not well established. Thus, further investigation of DMSO on more cell types especially the mechanisms responsible for the cytotoxic effect are needed. In order to make a general conclusion, in this study, we examined the effect of DMSO on two human suspension myeloid leukemia cell lines and two epithelia-derived adherent cancerous cell lines.

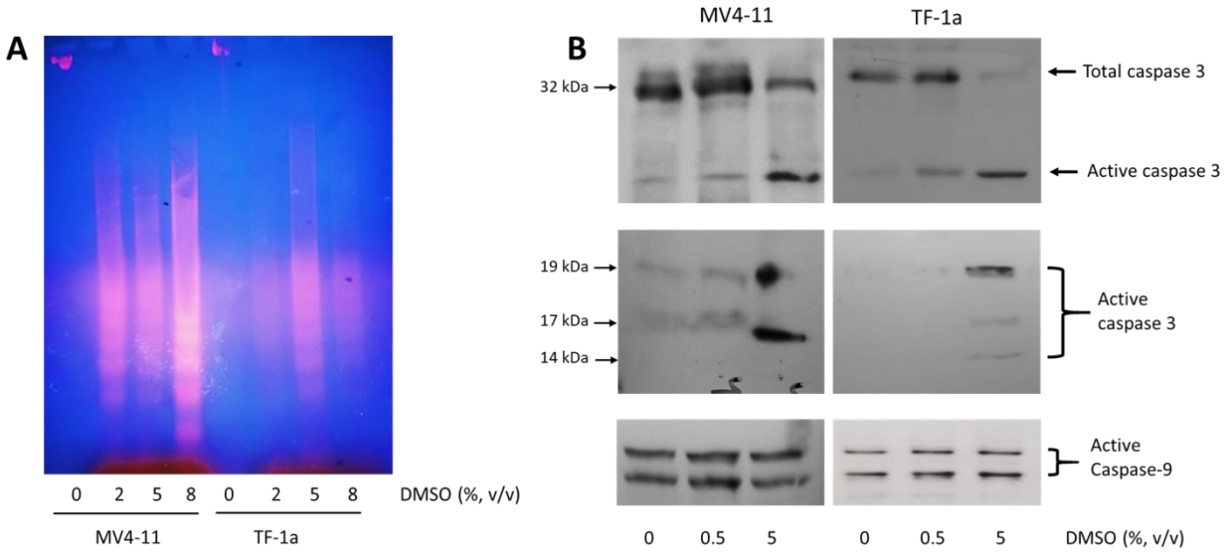

Fig. 4 DMSO causes DNA fragmentation. MV4-11 and TF-1a cells in log phase treated with or without DMSO for 24h were collected and apoptotic DNA fragments were detected by using the Quick Apoptotic DNA ladder detection kit with minor modifications (A). The expression of total caspase 3, active caspase 3, and active caspase 9, was immunoblotted by anti-total caspase 3 (top panel), active caspase 3 (middle panel), and caspase 9 (bottom panel) antibody, respectively and analyzed with Western blotting (B).

These cell lines represent earlier and later progenitor cells, respectively. MV4-11 is CD34 negative and is one of the latest progenitor cell lines. TF-1a cells are CD38-/CD34+ and the cell line is one of the earliest hematopoietic cell lines. For epithelia-derived cancer cells, MCF7 is a late

progenitor cell line with lower proliferation ability in colony formation, whereas HEP-G2 is an earlier progenitor cell line with stronger proliferation ability. We demonstrated that DMSO inhibited the growth of these four types of cells in culture measured by XTT assays and cytology analysis. Since the cell viability measured by trypan-blue assay is less than growth inhibition measured by XTT assay, it suggests that DMSO-induced growth inhibition is not a simple result of its cytotoxic effect. To address this issue, we first examined the expression of CDK2 and CDK2 complexes because CDKs and cyclins are the molecules common to both cell growth inhibition and apoptosis and CDK2 is a key regulator in the cell cycle control of the G1/S and S/G2 transitions (29). The activity of CDK2 is dependent on its association with cyclin A and cyclin E. In the G1/S phase, CDK4 and/or CDK6 in a complex with cyclin D initially phosphorylate the retinoblastoma (Rb) protein. Subsequently, the CDK2/cyclin E complex completes the phosphorylation of Rb, which releases and activates the E2F family transcriptional activity, driving cells to the S phase. In late S phase, CDK2-cyclin A phosphorylate several substrates, to control the cell cycle transition from S to G2. In addition, CDK2 can also directly phosphorylates p27Kip1 (CDK inhibitor) and induces p27Kip1 degradation through the proteasome pathway, which facilitates the cell cycle progression. In this study, we have detected that DMSO at 5% significantly inhibited the expression of CDK2 and cyclin A but not cyclin E. DMSO also downregulated CDK2-cyclin A and CDK2-cyclin E complexes. Since cell cycle inhibition is often linked to apoptosis, we next determined whether DMSO caused apoptosis in the cells tested. Our results demonstrated that the DMSO remarkably caused apoptosis in these cells, evidenced by following observations: (a) addition of DMSO to the cell culture inhibited the growth of MV4-11, TF-1a, Hep-G2, and MCF7 cells, measured by XTT assay; (b) cytology study showed some apoptotic changes in cell morphology including cell shrinkage, chromatin condensation, and breakages of nuclei; (c) the breakages of nuclei were confirmed to be DNA fragmentation, a key characteristic of cell apoptosis; and (d) Caspase 3 was activated in DMSO-treated cells but not in the control cells. The specificity of caspase 3 activation was confirmed by using a caspase inhibitor, zVAD-FMK, since the inhibitor at a concentration of 100 M completely inhibited DMSO-induced activation of caspase 3 in our preliminary studies (data not shown). Since DMSO had no marked effect on the expression of caspase 9, our results suggest that DMSO induced the apoptosis by the extrinsic signal pathway. In summary, DMSO not only has cytotoxic effects but also inhibited cell cycle regulatory molecules and activated apoptotic proteins. The data from this study provides supplementary information for using DMSO as a solvent, cryopreservation of cells, and clinical applications.

Conflicts Of Interest

None

Acknowledgments

This study was supported by Barry University Faculty Research Fund to Tang Hu.

References

1. Gurtovenko AA, Anwar J. Modulating the structure and properties of cell membranes: the molecular mechanism of action of dimethyl sulfoxide. J Phys Chem B. 2007; 111(35):10453-60.

2. Da Violante G, Zerrouk N, Richard I, Provot G, Chaumeil JC, Arnaud P. Evaluation of the cytotoxicity effect of dimethyl sulfoxide (DMSO)on Cac o2/TC7 colon tumor cell cultures. Biol Pharm Bull. 2002; 25(12):1600-03.
3. Kloverpris H, Fomsgaard A, Handley A, Ackland J, Sullivan M, GoulderP.Dimethylulfoxide (DMSO) exposure to human peri pheral blood mononuclear cells (PBMCs) abolish T cell responses only in high concentrations and following coincubation for more than two hours. J Immunol Methods. 2010; 356(1-2):70-8.
4. Shainhouse JZ, Grierson LM, Naseer Z. A long-term, open-label study to confirm the safety of topical diclofenac solution containing dimethyl sulfoxide in the treatment of the osteoarthritic knee. Am J Ther. 2010;17(6):566-76.
5. Zheng X, Gu Z, Huang Z, Ding H, Vasquez HE, Liu Y, Shi Y, Wang A. The effects of cryoprotectants on sperm motility of the Chinese pearl oyster. Cryobiology 2018; 82:64-9.
6. Sumida K, Igarashi Y, Toritsuka N, Matsushita T, Abe-Tomizawa K, Aoki M, Urushidani T, Yamada H, Ohno Y. Effects of DMSO on gene expression in human and rat hepatocytes. Hum Exp Toxicol. 2011;30(10):1701-9.
7. Yuan Y, Yang Y, Tian Y, Park J, Dai A, Roberts RM, Liu Y, Han X. Efficient long-term cryopreservation of pluripotent stem cells at -80 °C. Sci Rep. 2016; 6:34476. 13 pages.
8. Galvao, J., Davis B, Tilley M, Normando E, Duchen MR, Cordeiro MF. Unexpected low-dose toxicity of the universal solvent DMSO. FASEB J 2014; 28(3): 1317–30.
9. Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilization of dimethyl sulfoxide: pharmaco logical, cellular, and molecular aspects. Biochem Pharmacol. 2003;65(7):1035-41.
10. Swanson BN. (1985) Medical use of dimethyl sulfoxide (DMSO). Rev Clin Basic Pharm. 1985;5(1-2):1-33.
11. Li R, Johnson R, Yu G, McKenna DH, Hubel A. Preservation of cell-based immunotherapies for clinical trials. Cytotherapy. 2019; 21(9):943-957.
12. Franěk R, Marinović Z, Lujić J, Urbányi B, Fučíková M, Kašpar V, Pšenička M, Horváth Á. Cryopreservation and transplantation of common carp spermatogonia. PLoS One. 2019; 14(4):e0205481.
13. Kim LS, Axelrod LJ, Howard P, Buratovich N, Waters RF. Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: a pilot clinical trial. Osteoarthritis Cartilage. 2006;14(3):286-94
14. Hoang BX, Tran DM, Tran HQ, Nguyen PT, Pham TD, Dang HV, Ha TV, Tran HD, Hoang C, Luong KN, Shaw DG. Dimethyl sulfoxide and sodium bicarbonate in the treatment of refractory cancer pain. J Pain Palliat Care Pharmacother. 2011;25(1):19-24.
15. Rawls WF, Cox L, Rovner ES. Dimethyl sulfoxide (DMSO) as intravesical therapy for interstitial cystitis/bladder pain syndrome: A review. Neurourol Urodyn. 2017; 36(7):1677-1684.
16. García Rojo E, González Padilla DA, Castelo Loureiro A, Parrilla Rubio L, Tejido Sánchez Á, Guerrero Ramos F. Transl Androl Urol. 2019;8(5):548-55
17. Julien C, Marcouiller F Bretteville A, El Khoury NB Baillargeon J, He´bert SS, Planel E. Dimethyl Sulfoxide Induces Both Direct and Indirect Tau Hyperphosphorylation. PloS One 2012; 7(6) e40020.
18. Aita K, Irie H, Tanuma Y, Toida S, Okuma Y, Mori S, Shiga J. Apoptosis in murine lymphoid organs following intraperitoneal administration of dimethyl sulfoxide (DMSO). Exp Mol Pathol. 2005;79(3):265-71.
19. Singh M, McKenzie K, Ma X. Effect of dimethyl sulfoxide on in vitro proliferation of skin fibroblast cells. Journal of Biotech Research 2017; 8:78-82.
20. Liang C, Xue Z, Cang J, Wang H, Li P. (2011) Dimethyl sulfoxide induces heme oxygenase-1 expression via JNKs and Nrf2 pathways in human umbilical vein endothelial cells. Molecular and Cellular Biochemistry 2011; 355(1-2): 109-15.
21. Hajighasemi F, Tajik S. Assessment of cytotoxicity of dimethyl sulfoxide in human hematopoietic tumor cell lines. Iranian Journal of Blood & Cancer 2017; 9: 48-53.
22. de Abreu Costa L, Henrique Fernandes Ottoni M, Dos Santos MG, Meireles AB, Gomes de Almeida V, de Fátima Pereira W, Alves de Avelar-Freitas B, Eustáquio Alvim Brito-Melo G. Dimethyl Sulfoxide (DMSO) Decreases Cell Proliferation an d TNF-α, IFN-γ, and IL-2 Cytokines Production in Cultures of Peripheral Blood Lymphocytes. Molecules. 2017; 22(11):1789. 10 pages.
23. Gervais FG, Xu D, Robertson GS, et al. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation. *Cell*. 1999; 97 (3): 395–406.
24. Patel, T., Gores, G. J., and Kaufmann, S. H. The role of

proteases during apoptosis. FASEB J., 1996; 10: 587–97.

25. Jänicke RU, Sprengart ML, Wati MR, and Porter AG. Caspase-3 Is Required for DNA Fragmentation and Morphological Changes Associated with Apoptosis. J bio Chem 1998; 273(16):9357-9360.
26. Hu X, William E. Janssen, Lynn C. Moscinski, et al. An IκBα inhibitor causes apoptotic cell death via a p38 MAP kinase-dependent, NF- κ B-independent mechanism. *Cancer Research* 2001; 61: 6290-6296.
27. Yi X, Liu M, Luo Q , Zhuo H, Cao H , Wang J and Han Y. Toxic effects of dimethyl sulfoxide on red blood cells, platelets, and vascular endothelial cells in vitro. FEBS Open Bio. 2017; 7(4): 485–494.
28. Kang MH, Das J, Gurunathan S, Park HW, Song H, Park C, Kim JH. The cytotoxic effects of dimethyl sulfoxide in mouse preimplantation embryos: a mechanistic study. Theranostics. 2017;7(19):4735-52
29. Hu X , Zhang X, Zhong Q, Fisher AB, Bryington M, Zuckerman KS. Differential effects of transforming growth factor on cell cycle regulatory molecules in human myeloid leukemia cells. Oncogene 2001; 20:6840-50.