British Journal of Medicine & Medical Research
5(1): 65-74, 2015, Article no.BJMMR.2015.007
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

Low Concentration DMSO Stimulates Cell Growth and *In vitro* Transformation of Human Multiple Myeloma Cells

Jianguo Wen[1], Yijiu Tong[1] and Youli Zu[1*]

[1]Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, TX 77030, USA.

Authors' contributions

This work was carried out in collaboration between all authors. Author JW performed the experimental analysis, wrote the protocol, and wrote the first draft of the manuscript. Author YT performed the experimental analysis. Author YZ designed the study and managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/BJMMR/2015/5276
Editor(s):
(1) Salomone Di Saverio, Emergency Surgery Unit, Department of General and Transplant Surgery, S. Orsola Malpighi University Hospital, Bologna, Italy.
Reviewers:
(1) Anonymous, UCSI University, Malaysia.
(2) Anonymous, University of Toronto, Canada.
(3) Donard S. Dwyer, Departments of Psychiatry, and Pharmacology, Toxicology and Neuroscience, Louisiana State University Health Sciences Center, USA.
Peer review History: http://www.sciencedomain.org/review-history.php?iid=644&id=12&aid=5945

Original Research Article

Received 12th June 2013
Accepted 7th August 2013
Published 5th September 2014

ABSTRACT

Aims: To evaluate the effect of Dimethyl sulfoxide (DMSO) on multiple myeloma (MM) cells.
Study Design: Experimental study.
Place and Duration of Study: Department of Pathology and Genomic Medicine, the Methodist Hospital, Cancer Pathology Lab, the Methodist Hospital Research Institute, between 2011 and 2013.
Methodology: We treated RPMI 8226 and Dox-40 MM cells with DMSO. The cell growth, proliferation, apoptosis, and colony formation were examined.
Results: Exposure of RPMI 8226 and Dox-40 myeloma cells to low concentrations of DMSO resulted in a marked increase in cell growth as detected by viable cell counts and cell proliferation analysis. This DMSO-stimulated cell growth showed a dose-dependent pattern and could reach a maximal 3.57 fold-increase in the presence of 0.2% DMSO. In contrast, other common solvents

**Corresponding author: Email: yzu@houstonmethodist.org;*

including methanol and ethanol had little or no effect on cell growth. In addition, the *in vitro* cell transformation assay by colony formation in soft agar culture revealed that the presence of low concentrations of DMSO significantly enhanced potential of oncogenesis of myeloma cells.
Conclusion: Taken together, the findings demonstrate that DMSO could stimulate growth and the *in vitro* transformation of myeloma cells. However, further work is needed to understand the effect of DMSO on the pathogenesis and progression of MM.

Keywords: Cell growth; DMSO; in vitro transformation; multiple myeloma.

1. INTRODUCTION

Multiple myeloma is characterized by a clonal/malignant proliferation of plasma cells resulting in a serum monoclonal protein and multiple osteolytic lesions [1,2]. In 2011, approximately 20,500 individuals in the United States were newly diagnosed with multiple myeloma, and approximately 10,600 people died from multiple myeloma and its complications, accounting for approximately 20% of deaths from hematologic malignancies [3]. The American Cancer Society has estimated that 21,700 new cancer cases of MM will be diagnosed in the United States in 2012, and an estimated 10,710 deaths from the disease will occur [4].

The causes of multiple myeloma and the reasons for the racial disparity in incidence are unclear. Evidence from clinical studies suggests that exposures to certain biochemical hazards and organic solvents may be associated with increased incidence of MM [5-9]. In addition, individuals exposed to large amounts of radiation, such as survivors of the atomic bomb explosions in Japan, have an increased risk for myeloma [10]. Moreover, genetic factors, low socioeconomic status (SES), obesity, viruses and other infectious agents have been implicated [11-13]. Recent studies also suggest that certain cytokines and chromosomal abnormalities may be involved in the pathogenesis of multiple myeloma [14-18].

DMSO ($(CH3)_2SO$), first identified in the 19th century as a byproduct of the paper and wood industries, is a polyfunctional molecule and one of the most widely used solvents in organic chemistry, chemical technology, and cell biology [7-9,19-22]. It is also used as cryoprotectant reagent in the freezing preservation of cells and tissues [23]. In addition, its biological functions include producing cell fusion, inducing cell differentiation, increasing permeability across cell membranes, and functioning as a free radical scavenger [24]. Human investigation of DMSO was suspended because of appearance of changes in the refractive index of the eye in experimental animal [25]. Oral LD50 values (ie, doses resulting in 50% mortality rates) of between 4 and 29 g/kg after acute dosing have been reported in a variety of laboratory animals, including rodents, dogs, and primates [22]. However, with the increasing data about the favorable safety profile, the clinical investigation of DMSO has not been specifically regulated by FDA since 1980 [22]. In medical fields DMSO is used for anesthesia [26], anti-inflammation effect, antiviral and antibacterial activity and radioprotection abilities [27], and to protect against drug-induced tissue necrosis after paravasation [17,21]. Recent studies of myeloma and leukemia cells showed that DMSO can potentiate the death receptor ligand-induced apoptosis [28]. DMSO can also activate tumor necrosis factoralpha-p53 mediated apoptosis and down regulates D-fructose-6-phosphate-2-kinase and lactate dehydrogenase-5 in Dalton's lymphoma in vivo [29]. However, further research is needed to evaluate the effect of DMSO on myeloma cells.

To investigate the role of DMSO in myeloma, we utilized human myeloma cell lines RPMI 8226 and its variant Dox40 for this study. Myeloma cells were exposed to low concentrations of DMSO, and resultant changes in cell growth, proliferation, and apoptosis were examined. In addition, the effect of DMSO on *in vitro* transformation and on cogenesis of myeloma cells was investigated using a soft agar colony formation assay.

2. MATERIALS AND METHODS

2.1 Cell Lines and Culture Reagents

Multiple myeloma cell lines: The human myeloma cell lines RPMI 8226and its doxorubicin-resistant variant Dox-40 cells were provided kindly by Dr. William Dalton [30-32]. The cells were routinely maintained in RPMI 1640 medium containing 5%

fetal calf serum (FCS). Reagents: RPMI 1640 medium, FCS, and molecular biology grade DMSO, ethanol, methanol and other cell culture reagents are purchased from Sigma Aldrich (St. Louis, MO).

2.2 Cellular Functional Assays

For cell growth assay, RPMI 8226and Dox-40 myeloma cells (5x 10^4cells/ml) were cultured in RPMI 1640 medium with 2% FCS. Cells were treated with or without DMSO, methanol, or ethanol as indicated for each experiment in 12-well culture plates. After treatment as specified, cells were harvested and stained with 0.1% trypan blue for 15 minutes at room temperature. Dead cells were stained and viable cells with intact cell membrane were transparent under light microscope. To evaluate cell growth the viable cells were counted using a hemocytometer with light microscopy. The number from three separate wells was counted. To study the dose effect of DMSO the cultured myeloma cells were exposed to different concentration of DMSO, methanol, and ethanol, from 0 to 1.6% of final concentration as indicated in each experiment. In addition, to know serum effect on DMSO induced-cellular functions the cells were cultured in RPMI 1640 with 1 to 10% of FCS and treated with 0.2% DMSO.

2.3 MTT Cell Proliferation Assay

RPMI 8226 myeloma cells (5x10^4 cells/ml) were cultured in RPMI1640 with 2% FCS and treated with different concentration of DMSO as indicated for 24 hours. The cultured cells (100 µl/sample) were transferred to a 96-wells plate and incubated with 10 µl of AB solution of MTT assay (Chemicon International, Temecula, CA) at 37ºC for 4 hours. Cells were then lysed by addition of 100 µl Detergent Reagent. Supernatants of cell lysates were analyzed by BioRad (Hercules, CA) microplate reader and changes of absorbance at 570 nm were recorded with a reference wavelength of 630 nm. Final results are a mean number from 3 samples/experiment and each experiments were repeated ≥ 3 times with similar results, as previously reported [32].

2.4 Cell Apoptosis Assay

RPMI 8226 myeloma cells (1x10^5 cells/ml) were cultured in RPMI 1640 with 2% FCS and treated with or without different concentration of DMSO as indicated for 24 hours. The treated cells were harvested, fixed in 100 µl of binding buffer, and stained with 5 µl of FITC-conjugated Annexin V and 5 µl of propidium iodide (PI) (BD Biosciences, San Jose, CA) for 15 min at room temperature in dark. After addition of 400 µl of binding buffer, the apoptotic cells were detected with a four-color flow cytometry and analyzed by FACSDiva software (BD Biosciences), as previously reported [32,33]. Early apoptotic cells staining with Annexin V-FITC, but not PI, appear in the lower right (LR) quadrant of data plots. Late apoptotic cells appear in the upper right (UR) quadrant, staining with both PI and annexin V-FITC. Both early apoptotic (Annexin V-positive, PI-negative) and late (Annexin V-positive and PI-positive) apoptotic cells were included in cell death determinations [34].

2.5 Soft Agar Cell Colony Formation Assay

The *in vitro* cell colony formation assay is a measure of anchorage independent growth in soft agar, and is considered the most stringent *in vitro* assay for detecting potential of cell malignant transformation [35,36]. It is a quantitative test and also allows screening of potential substances that regulate cell malignant transformation. To make soft agar cultures 4x10^3 RPMI 8226 myeloma cells were suspended in 0.5 ml of 0.4% soft agar (DIFCO Bactoagar, Detroit, MI) in RPMI 1640 medium with 2% FCS at 37ºC. After gently mixing the cell/soft agar suspension were overlaid onto the previously prepared bottom agar layer in a single well of a 12-wells tissue culture plate. The bottom agar layer was prepared 1 day prior to being used and contained 1.5 ml of 0.8% agar/well in RPMI 1640 medium with 2% FCS. After incubation for 24 hours at 37ºC in a 5% CO2 incubator the top cell/soft agar layer was formed and 0.5 ml of fresh RPMI 1640 medium containing 2% FCS with or without DMSO was then added to surface of the soft agar layer. The culture medium was replaced every 3 days and cell colony formation was monitored by microscopic examination once a day for 12 days. To count the formed cell colonies cells in soft agar were stained with 0.5 ml of cell staining solution overnight (Chemicon International). The stained cell colonies were photographed using a digital camera. The formed cell colonies in individual wells were counted and the experiment was repeated at least three times. Final results were reported as the average number of cell colonies per well from three wells for each condition.

2.6 Flow Cytometric Evaluation of the Activation of ERK ½

Cultured RPMI 8226myeloma cells were exposed to DMSO at different concentration from 0.1 to 1.0% as described above for 30 minutes. The cells were fixed, permeabilized, and the activated cellular ERK1/2 was probed by a PE-conjugated monoclonal antibody (BD Biosciences) specific for the phosphorylated form of the kinase. The phosphorylated/activated form of cellular ERK1/2 was detected by flow cytometry analysis, as previously reported [32].

2.7 Statistical Analysis

The data represent the mean ± SD of 3 independent experiments. Statistical differences between experimental groups were analyzed with the software SPSS version 11.5, using *t* test or multiple comparison test after ANOVA (*, $P<0.05$; **, $P<0.01$).

3. RESULTS

3.1 The Presence of Low Concentrations of DMSO Enhanced Cell Growth of Cultured Human Multiple Myeloma Cells

To study the cellular effect of DMSO theRPMI 8226 myeloma cells were cultured in RPMI 1640 medium supplemented with 2% FCS and exposed to low level of DMSO ranging from 0.05% to 1.6% final concentration as indicated in Fig. 1A. After culture for 5 days the cells were stained by 0.1% trypan blue and viable cells were counted under light microscope using a hemocytometer. The relative cell growth rate is shown in comparison to control cultures (without DMSO exposure). The presence of DMSO enhanced RPMI 8226 myeloma cell growth and this effect appears to be dose-dependent. The enhanced growth was noted at the lowest concentration tested (0.05% DMSO) and reached a maximum of 3.57 fold-increase in the presence of 0.2% DMSO. The improved growth rate disappeared when DMSO concentrations were higher than 0.8% (Fig. 1A). To confirm this observation, Dox-40 myeloma cells (a Doxorubicin-resistant variant of RPMI 8226 myeloma cell line) were also used and treated with DMSO. As shown in Fig. 1B, the presence of a low level of DMSO enhanced Dox-40 myeloma cell growth in a similar dose-dependent and bell-shaped fashion, but to a lesser extent. A maximum of 2.14 fold-increase was observed when 0.2% DMSO was included. These findings demonstrate that the presence of low levels of DMSO could stimulate cell growth of human multiple myeloma cells.

To further explore this phenomenon, RPMI 8226 myeloma cells were cultured with medium containing different concentration of FCS ranging from 1.25% to 10% as shown in Figure 2. The cells ($5x10^4$/ml) were exposed to 0.2% DMSO for 3 days and changes in cell growth was evaluated by counting viable cells using trypan blue staining. The relative cell growth rate was compared to control cultures without DMSO treatment. In Fig. 2, open bars represent cell numbers from cultures without DMSO and solid bars represent cell numbers from culture with 0.2% DMSO. The DMSO-enhanced cell growth was most evident in culture containing 1.25% FCS with a 1.9-fold increase. While a 1.1-fold increase was seen in culture containing 2.5% FCS, 0.59-fold in 5% FCS, and a 0.53-fold increase in 10% FCS, suggesting DMSO has an improved ability to stimulate myeloma cell growth under low serum conditions.

In addition to DMSO, two other common organic solvents, ethanol and methanol, were tested. RPMI 8226 myeloma cells were exposed for 3 days to methanol or ethanol at a final concentration ranging from 0.05 to 0.8% and viable cell number was counted as described above. As shown in Fig. 3, in contrast to DMSO the presence of methanol and ethanol in the cultures had little to no effect (the largest difference seen was a 0.16-fold increase with 0.2% ethanol) on RPMI 8226 myeloma growth.

To validate the observed DMSO effect on myeloma cell proliferation the cultured RPMI 8226 myeloma cells were exposed to DMSO, methanol, or ethanol at final concentration of 0.05 to 0.8% as indicated for 24 hour and MTT cell proliferation assay was performed as described under “Material and Methods” [32]. Fig. 4 indicates that with the MTT assay the presence of DMSO stimulates proliferation of RPMI 8226 myeloma cells in a manner similar to that detected by viable cell number in Fig. 3. Exposure of RPMI 8226 myeloma cells to ethanol or methanol did not stimulate cell proliferation by MTT assay.

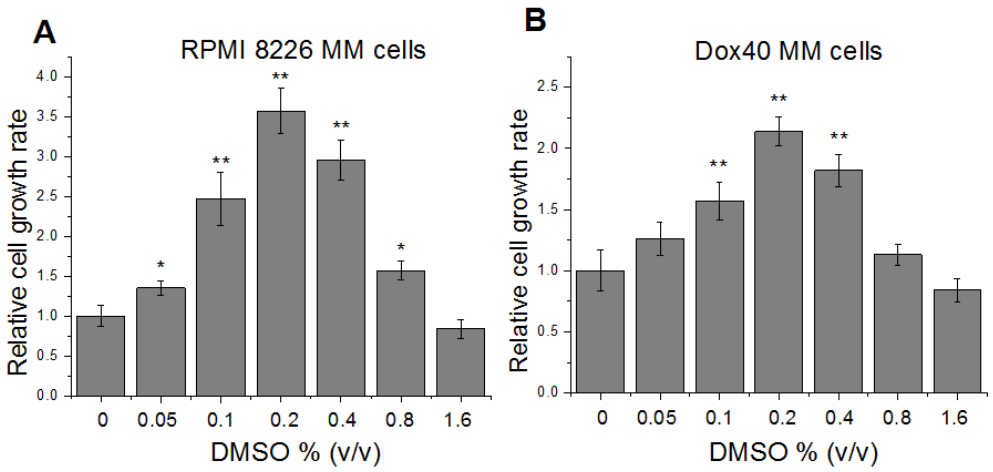

Fig. 1. Low concentrations of DMSO enhance growth of RPMI 8226 and Dox-40 myeloma cells in a dose-dependent fashion

A, *RPMI 8226 myeloma cells were cultured in the presence of different concentrations of DMSO as indicated with 2% FCS. After 5 days the cells were stained by trypan blue and the number of viable cells counted under light microscope with hemocytometer. The relative cell growth rate was determined by dividing the cell number by the number of cells counted for the control (cells cultured without DMSO).* ***B,*** *Dox-40 myeloma cells (doxorubicin-resistant variants of RPMI 8226 myeloma cells) were treated with different concentrations of DMSO for 5 days as indicated with 2% FCS and relative cell growth rate was evaluated. The relative cell growth rate was determined by dividing the cell number by the number of cells counted for the control (cells cultured without DMSO). The data represent the mean ± SD of 3 independent experiments. *, P < 0.05; **, P < 0.01 compared with control (cells cultured without DMSO). Statistical significance was determined by multiple comparison test after ANOVA.*

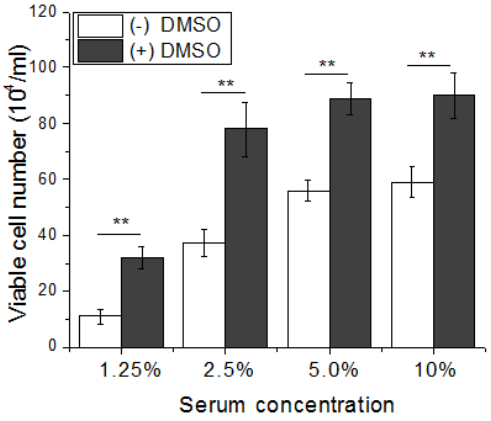

Fig. 2. Enhanced DMSO effects on cell growth when cultured in low FCS concentrations

*RPMI 8226 myeloma cells were cultured in different concentrations of FCS as indicated and treated with 0.2% DMSO for 3 days. The cells were stained by trypan blue and number of viable cells was counted under microscope using a hemocytometer. Open bars represent cell numbers from cultures without DMSO. Solid bars represent the cell numbers from cultures with 0.2% DMSO. The data represent the mean ± SD of 3 independent experiments. *, P < 0.05; **, P < 0.01 compared with control (cells cultured without DMSO). Statistical significance was determined by t test*

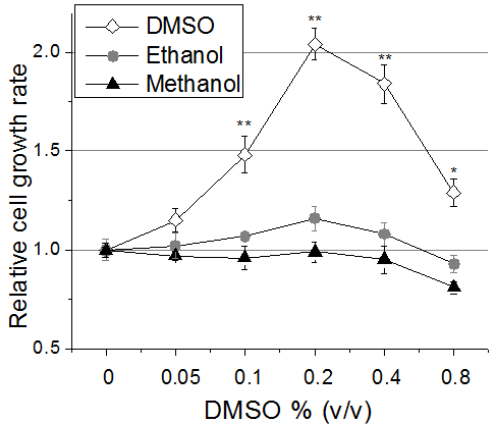

Fig. 3. Methanol and ethanol had little effect on RPMI 8226 myeloma cell growth

*RPMI 8226 cells were cultured in 2% FCS and exposed to methanol, ethanol, and DMSO at a final concentration of 0.05-0.8% as indicated. After culture for 3 days the cells were stained by trypan blue and number of viable cells was counted under microscope with hemocytometer. Relative cell growth rates were obtained by dividing cell numbers with exposure by cell numbers obtained in control cultures without treatment. The data represent the mean ± SD of 3 independent experiments. *, P < 0.05; **, P < 0.01 compared with control (cells cultured without treatment). Statistical significance was determined by multiple comparison test after ANOVA*

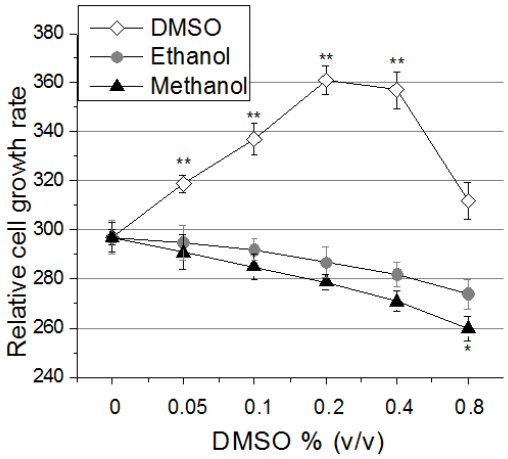

Fig. 4. DMSO enhanced RPMI 8226 myeloma cell proliferation, but not methanol and ethanol

*RPMI 8226 myeloma cells were cultured in 2% FCS and exposed to DMSO, methanol, and ethanol for 24 hours at a final concentration of 0.05-0.8% as indicated. The cultured cells were harvested and their relative proliferation/growth rate was detected by a MTT assay as described under "Material and Methods". The relative proliferation rate of myeloma cells was showed by absorbance at OD570. The data represent the mean ± SD of 3 independent experiments. *, P < 0.05; **, P < 0.01 compared with control (cells cultured without treatment). Statistical significance was determined by multiple comparison test after ANOVA*

3.2 DMSO Enhanced Potential of *In vitro* Transformation of RPMI 8226 Myeloma Cells

To investigate effects of DMSO on potential of myeloma cell transformation the cultured RPMI 8226 myeloma cells (8x103 cells/ml) were seeded into soft agar medium as described under "Material and Methods". The cells within soft agar were exposed to low concentrations of DMSO ranging from 0.1 to 0.8% for 12 days. The formed cell colonies within soft agar culture wells were stained as shown in Figs. 5A and 5B. The total number of the formed cell colonies per well were counted under microscope. The DMSO effect on in vitro transformation of RPMI 8226 myeloma cells was determined by cell colony formation index (colony number with DMSO treatment/colony number in DMSO (-) control). As shown in Fig. 5C the presence of a low level of DMSO enhanced the in vitro transformation/colony formation of RPMI 8226 myeloma cells in a dose-dependent fashion. A maximal increase of 2.63-fold was observed with exposure to 0.4% DMSO. It is different from its effect on cell growth, in which maximal effect is seen in 0.2% DMSO. This difference in effect between cell growth (0.2% DMSO) and in vitro colony formation (0.4% DMSO) may be due to the ability of DMSO to be dispersed within soft agar resulting in lower local concentration levels.

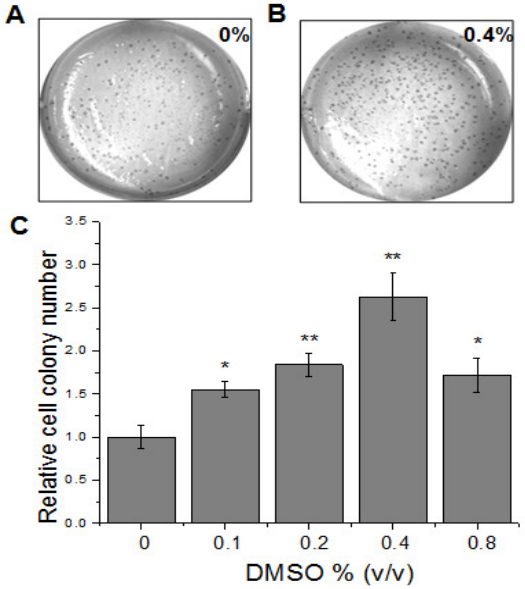

Fig. 5. DMSO enhanced the *in vitro* colony formation of RPMI 8226 myeloma cells in a soft agar culture

*To examine the potential of in vitro transformation of myeloma cells, soft agar cell colony formation assays were performed. RPMI 8226 myeloma cells were seeded into soft agar medium and cultured with 2% FCS for 12 days in the presence of DMSO at a final concentration of 0.1-0.8% as described under "Material and Methods". The soft agar cultures were stained with cell staining solution over night and photographed: **A,** cell colony formation in control culture without DMSO and **B,** cell colony formation in culture with 0.4% DMSO. The number of the formed cell colonies following exposure to different concentration of DMSO was counted using a light microscope. The relative in vitro cell colony formation is graphed in **C**. The data represent the mean ± SD of 3 independent experiments.*
**, P < 0.05; **, P < 0.01 compared with control (cells cultured without DMSO). Statistical significance was determined by multiple comparison test after ANOVA.*

3.3 Low Concentrations of DMSO had a Minimal Effect on Cell Apoptosis of RPMI 8226 Myeloma Cells

To test whether low levels of DMSO had any effect on cell apoptosis RPMI 8226 myeloma cells were cultured in 2% FCS and exposed to DMSO at different concentration (0.1-0.4%) for 2 days. Cells were then stained with FITC-conjugated Annexin V and PI as described under "Material and Methods". The apoptotic cells rate (%) was determined by flow cytometry analysis of Annexin V and PI. As shown in Fig. 6, the

presence of low concentration of DMSO resulted in a slight increase in apoptosis of RPMI 8226 myeloma cells. The control cultures (without DMSO) yielded an apoptotic rate of 9.43%, compared to a 10.57% apoptotic rate in cultures with 0.1% DMSO, a 12.14% apoptotic rate with 0.2% DMSO, and a 13.3% apoptotic rate in cultures with 0.4% DMSO. There was no statistically significant difference between the low concentration of DMSO treatment and control (without DMSO).

4. CONCLUSION AND DISCUSSION

Exposure of U937 and HL-60 leukemia cells to DMSO alone had little or a minimal effect on cell apoptosis [28,37-39]. Moreover, DMSO did not affect expression of proteins in death signal transduction, such as Bcl-2 family proteins, FADD, caspase-3 and -8, the inhibitor of apoptosis proteins (IAPs) or cFLIPL [28]. Our study also indicates that with RPMI 8226myeloma cells and its variant Dox-40 myeloma cells a low level of DMSO results in a small effect in cell apoptosis (no statistically significant difference). In contrast, some studies demonstrate that DMSO can cause the cytotoxicity [20,40]. However, the concentration of DMSO they used is 2% or 5% which is much higher than the highest concentration (0.8%) used in our study. Thus, we believe that the concentration of DMSO is critical to determine its function.

Interestingly, in the meantime of the low level of DMSO-induced apoptosis, we observed the DMSO-enhanced cell proliferation as measured by MTT assay and DMSO-improved cell growth as measured by viable cell counting with trypan blue staining. In recent study, it was shown that tumor cells that were induced to die by radiotherapy stimulate tumor regrowth [41]. Dying tumor cells were able to secrete signaling molecules for tumor cells proliferation. Caspase-3 which was activated during apoptosis was required to mediate this stimulation through cleavage and activation of cytosolic calcium-independent phospholipase A2 (iPLA2) that ultimately produces prostaglandin E2 [41,42]. Therefore, radio-and chemotherapy-induced apoptotic tumor cells can induce proliferation of surviving tumor cells, which may dampen the therapy effect [43]. In addition, we found that the presence of DMSO augmented *in vitro* transformation/colony formation of RPMI 8226 myeloma cells. Taken together, our findings demonstrate that low levels of DMSO have an overall stimulating effect on *in vitro* cell growth and potential of transformation of RPMI 8226myeloma cells.

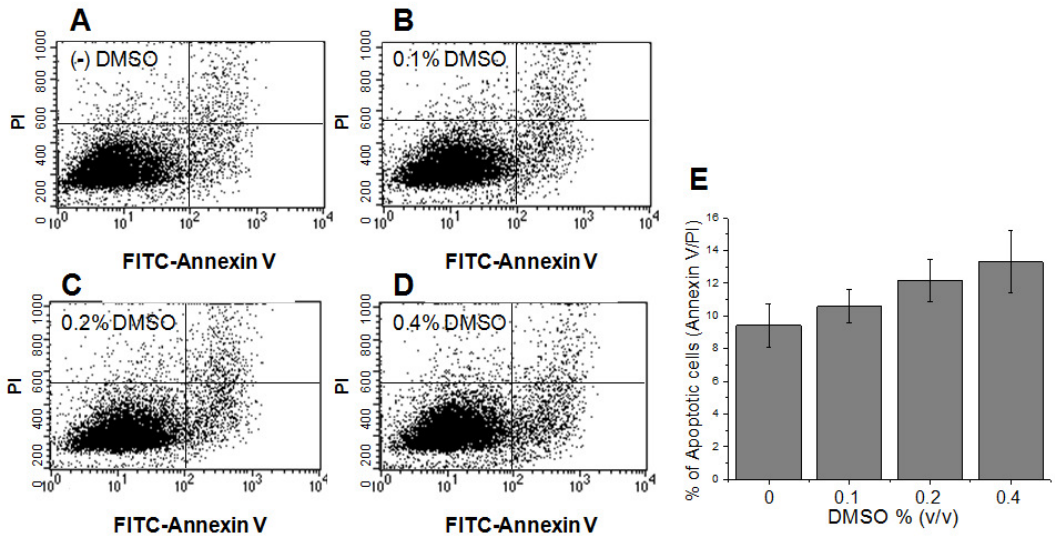

Fig. 6. Low levels of DMSO had a minimal effect on apoptosis of RPMI 8226 myeloma cells

*The cells were cultured in 2% FCS and exposed to DMSO for 24 hours at a final concentration of 0% (**A**), 0.1% (**B**), 0.2% (**C**),and 0.4% (**D**). The cultured cells were then stained with FITC-conjugated Annexin V and PI and apoptotic cells were detected by flow cytometry analysis as described under "Material and Methods". The percentage of apoptosis is graphed in **E**. The data represent the mean ± SD of 3 independent experiments. No significant difference between experimental groups and control (cells cultured without DMSO). Statistical significance was determined by multiple comparison test after ANOVA*

The activation of cellular ERK1/2 has been reported to play a critical role in myeloma cell growth and proliferation [44,45]. To study molecular mechanism of the DMSO stimulating-effect on myeloma cells we have investigated kinase activity of cellular ERK1/2. In RPMI 8226 myeloma cells ERK1/2 show basal activity and exposure of cells to DMSO did not alter the basal kinase activity (data not shown), indicating that ERK signaling pathway is not involved in the DMSO-induced proliferation of myeloma cells. Further studies will be needed to tease out the molecular mechanism (i.e., antioxidant activity) that is involved in the low-level DMSO increase in cell growth.

It has been reported that exposure to biohazards, radiation, and virus may be part of pathogenesis of plasma cell neoplasm [5,46]. Our studies indicate that DMSO shows more potential to enhance myeloma cell growth than either methanol or ethanol (Figs. 3 and 4). Findings from this *in vitro* study raise the question of whether low levels of DMSO could be an environmental cause factor for myeloma development. Our plan for the future is to examine the effect of DMSO in MM tumor-bearing mice model and this will help to strengthen our current findings and determine the potential role of DMSO in MM.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

We thank Dr. William Dalton for kindly providing myeloma cell lines. This study was partially supported by NIH grants 1R33CA173382, R01CA151955, and R21RCA150109.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Smith D, Yong K. Multiple myeloma. BMJ. 2013;346:f3863.
2. Anderson KC, Alsina M, Bensinger W, Biermann JS, Cohen AD, Devine S, et al. Multiple myeloma, version 1.2013. J Natl Compr Canc Netw. 2013;11(1):11-17.
3. Warren JL, Harlan LC, Stevens J, Little RF, Abel GA. Multiple myeloma treatment transformed: a population-based study of changes in initial management approaches in the United States. J Clin Oncol. 2013;31(16):1984-1989.
4. Siegel R, Naishadham D, Jemal A. Cancer statistics.CA Cancer J Clin. 2012;62(1):10-29.
5. Speer SA, Semenza JC, Kurosaki T, Anton-Culver H. Risk factors for acute myeloid leukemia and multiple myeloma: a combination of GIS and case-control studies. J Environ Health. 2002;64(7):9-16; quiz 35-16.
6. Costantini AS, Benvenuti A, Vineis P, Kriebel D, Tumino R, Ramazzotti V, et al. Risk of leukemia and multiple myeloma associated with exposure to benzene and other organic solvents: evidence from the Italian Multicenter Case-control study. Am J Ind Med. 2008;51(11):803-811.
7. Gold LS, Stewart PA, Milliken K, Purdue M, Severson R, Seixas N, et al. The relationship between multiple myeloma and occupational exposure to six chlorinated solvents. Occup Environ Med. 2011;68(6):391-399.
8. Perrotta C, Staines A, Codd M, Kleefeld S, Crowley D, A TM, et al. Multiple Myeloma and lifetime occupation: results from the EPILYMPH study. J Occup Med Toxicol. 2012;7(1):25.
9. Perrotta C, Kleefeld S, Staines A, Tewari P, De Roos AJ, Baris D, et al. Multiple myeloma and occupation: a pooled analysis by the International Multiple Myeloma Consortium. Cancer Epidemiol. 2013;37(3):300-305.
10. Preston DL, Kusumi S, Tomonaga M, Izumi S, Ron E, Kuramoto A, et al. Cancer incidence in atomic bomb survivors. Part III. Leukemia, lymphoma and multiple myeloma, 1950-1987. Radiat Res. 1994;137(Suppl-2):S68-97.
11. Rettig MB, Ma HJ, Vescio RA, Pold M, Schiller G, Belson D, et al. Kaposi's sarcoma-associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma patients. Science. 1997;276(5320):1851-1854.
12. Konrad RJ, Kricka LJ, Goodman DB, Goldman J, Silberstein LE. Brief report:

myeloma-associated paraprotein directed against the HIV-1 p24 antigen in an HIV-1-seropositive patient. N Engl J Med. 1993;328(25):1817-1819.
13. Hallek M, Bergsagel PL, Anderson KC. Multiple myeloma: increasing evidence for a multistep transformation process. Blood. 1998;91(1):3-21.
14. Durie BG. Cellular and molecular genetic features of myeloma and related disorders. Hematol Oncol Clin North Am. 1992;6(2):463-477.
15. Lai JL, Zandecki M, Mary JY, Bernardi F, Izydorczyk V, Flactif M, et al. Improved cytogenetics in multiple myeloma: a study of 151 patients including 117 patients at diagnosis. Blood. 1995;85(9):2490-2497.
16. Sawyer JR, Waldron JA, Jagannath S, Barlogie B. Cytogenetic findings in 200 patients with multiple myeloma. Cancer Genet Cytogenet. 1995;82(1):41-49.
17. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science. 1998;281(5381):1305-1308.
18. Konigsberg R, Zojer N, Ackermann J, Kromer E, Kittler H, Fritz E, et al. Predictive role of interphase cytogenetics for survival of patients with multiple myeloma. J Clin Oncol. 2000;18(4):804-812.
19. Santos NC, Martins-Silva J, Saldanha C. PTEN "meets" DMSO. Leuk Res. 2005;29(4):361-362.
20. Wang CC, Lin SY, Lai YH, Liu YJ, Hsu YL, Chen JJ. Dimethyl sulfoxide promotes the multiple functions of the tumor suppressor HLJ1 through activator protein-1 activation in NSCLC cells. PLoS One. 2012;7(4):e33772.
21. Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. Biochem Pharmacol. 2003;65(7):1035-1041.
22. Marren K. Dimethyl sulfoxide: an effective penetration enhancer for topical administration of NSAIDs. Phys Sportsmed. 2011;39(3):75-82.
23. Pegg DE. Principles of cryopreservation. Methods Mol Biol. 2007;368:39-57.
24. Capriotti K, Capriotti JA. Dimethyl sulfoxide: history, chemistry, and clinical utility in dermatology. J Clin Aesthet Dermatol. 2012;5(9):24-26.
25. Rubin LF. Toxicologic update of dimethyl sulfoxide. Ann N Y Acad Sci. 1983;411:6-10.
26. Jacob SW, Herschler R. Pharmacology of DMSO. Cryobiology. 1986;23(1):14-27.
27. Milligan JR, Ward JF. Yield of single-strand breaks due to attack on DNA by scavenger-derived radicals. Radiat Res. 1994;137(3):295-299.
28. Vondracek J, Soucek K, Sheard MA, Chramostova K, Andrysik Z, Hofmanova J, et al. Dimethyl sulfoxide potentiates death receptor-mediated apoptosis in the human myeloid leukemia U937 cell line through enhancement of mitochondrial membrane depolarization. Leuk Res. 2006;30(1):81-89.
29. Koiri RK, Trigun SK. Dimethyl sulfoxide activates tumor necrosis factoralpha-p53 mediated apoptosis and down regulates D-fructose-6-phosphate-2-kinase and lactate dehydrogenase-5 in Dalton's lymphoma in vivo. Leuk Res. 2011;35(7):950-956.
30. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Kung AL, Davies FE, et al. Antimyeloma activity of heat shock protein-90 inhibition. Blood. 2006;107(3):1092-1100.
31. Pellat-Deceunynk C, Amiot M, Bataille R, Van Riet I, Van Camp B, Omede P, et al. Human myeloma cell lines as a tool for studying the biology of multiple myeloma: a reappraisal 18 years after. Blood. 1995;86(10):4001-4002.
32. Wen J, Feng Y, Bjorklund CC, Wang M, Orlowski RZ, Shi ZZ, et al. Luteinizing Hormone-Releasing Hormone (LHRH)-I antagonist cetrorelix inhibits myeloma cell growth in vitro and in vivo. Mol Cancer Ther. 2011;10(1):148-158.
33. Wen J, Cheng HY, Feng Y, Rice L, Liu S, Mo A, et al. P38 MAPK inhibition enhancing ATO-induced cytotoxicity against multiple myeloma cells. Br J Haematol. 2008;140(2):169-180.
34. Gao N, Rahmani M, Dent P, Grant S. 2-Methoxyestradiol-induced apoptosis in human leukemia cells proceeds through a reactive oxygen species and Akt-dependent process. Oncogene. 2005;24(23):3797-3809.
35. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. Nature. 1999;400(6743):464-468.

36. Wen J, Feng Y, Huang W, Chen H, Liao B, Rice L, et al. Enhanced antimyeloma cytotoxicity by the combination of arsenic trioxide and bortezomib is further potentiated by p38 MAPK inhibition. Leuk Res. 2010;34(1):85-92.
37. Depraetere S, Vanhaesebroeck B, Fiers W, Willems J, Joniau M. Polar agents with differentiation inducing capacity potentiate tumor necrosis factor-mediated cytotoxicity in human myeloid cell lines. J Leukoc Biol. 1995;57(1):141-151.
38. Ohashi M, Iwase M, Nagumo M. Changes in susceptibility to Fas-mediated apoptosis during differentiation of HL-60 cells. J Leukoc Biol. 2000;67(3):374-380.
39. Vondracek J, Sheard MA, Krejci P, Minksova K, Hofmanova J, Kozubik A. Modulation of death receptor-mediated apoptosis in differentiating human myeloid leukemia HL-60 cells. J Leukoc Biol. 2001;69(5):794-802.
40. Teraoka H, Mikoshiba M, Takase K, Yamamoto K, Tsukada K. Reversible G1 arrest induced by dimethyl sulfoxide in human lymphoid cell lines: dimethyl sulfoxide inhibits IL-6-induced differentiation of SKW6-CL4 into IgM-secreting plasma cells. Exp Cell Res. 1996;222(1):218-224.
41. Huang Q, Li F, Liu X, Li W, Shi W, Liu FF, et al. Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. Nat Med. 2011;17(7):860-866.
42. Li F, Huang Q, Chen J, Peng Y, Roop DR, Bedford JS, et al. Apoptotic cells activate the "phoenix rising" pathway to promote wound healing and tissue regeneration. Sci Signal. 2010;3(110):ra13.
43. Ryoo HD, Bergmann A. The role of apoptosis-induced proliferation for regeneration and cancer. Cold Spring Harb Perspect Biol. 2012;4(8):a008797.
44. Chauhan D, Anderson KC. Mechanisms of cell death and survival in multiple myeloma (MM): Therapeutic implications. Apoptosis. 2003;8(4):337-343.
45. Platanias LC. Map kinase signaling pathways and hematologic malignancies. Blood. 2003;101(12):4667-4679.
46. Eriksson M, Karlsson M. Occupational and other environmental factors and multiple myeloma: a population based case-control study. Br J Ind Med. 1992;49(2):95-103.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=644&id=12&aid=5945