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Short Communication

The influence of dimethyl sulfoxide (DMSO) on metabolic activity and morphology of melanoma cell line WM-266-4

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Abstract: Dimethyl sulphoxide (DMSO) is widely used as a solvent in biomedical research, regularly in concentrations up to 1%. Nevertheless, little is known about the effect of different DMSO concentration on WM-266-4 metastatic melanoma cells, which are often used in melanoma research. Due to resistance of melanoma cells high concentrations of cytotoxic substances soluble in DMSO are used *in vitro* tests. Consequently, total DMSO concentration often exceeds 1%. The aim of our study was to test the metabolic activity and morphology of WM-266-4 cells exposed to selected DMSO concentrations for different period of time. Cells were incubated in selected ethanol concentrations for comparison. MTT test was performed to determine proliferation activity of the cells and morphological analysis was carried out by phase-contrast microscopy. Our results show inhibitory effect of DMSO on WM-266-4 cells' metabolic activity. Morphology of the cells changed progressively with the time of exposure. Ethanol showed little effect on metabolic activity of the cells and no effect on cell morphology after selected period of time. According to our study, for specific *in vitro* tests concentrations of DMSO up to 1.5% may be appropriate for WM-266-4 cell line experiments.

Key words: Dimethyl sulphoxide; Melanoma cell line; Metabolic activity; Morphology; MTT.

Introduction

Dimethyl sulphoxide (DMSO), an amphipatic solvent soluble in both water and organic substances, is often used to dissolve hydrophobic substances in biomedical research (1). However, DMSO was reported to interact with the metabolism and membrane of cells, resulting in cell damage (2, 3). DMSO concentrations up to 1% are generally used for *in vitro* tests (4-6). Nevertheless, little is known about the effect of different DMSO concentration on WM-266-4 cells. WM-266-4 is a metastatic human melanoma cell line with small flat mesenchymal morphology. The key applications of this cell line include various biological assays, drug testing and genetic studies (7, 8). Melanoma cells are known for their resistance (9-11). Due to the need to test high concentrations of substances insoluble in water and potentially cytotoxic on melanoma cells, different concentrations and times of exposure of DMSO to selected melanoma cell line were tested. Since ethanol is another commonly used solvent in cell based assays (12), we also tested selected concentration of ethanol for comparison. We focused on changes in metabolic activity and cell morphology of WM 266-4 cell line after exposure to DMSO and ethanol.

Materials and Methods

Cell culture

Melanoma cell line WM-266-4 (ATCC® CRL1676™) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were grown in complete medium containing Eagle's Minimum Essential Medium (EMEM, ATCC® 30-2003™) with 1% fetal bovine serum (FBS) (ATCC® 30-2021™) and 0.02% MycoZap™ Plus-CL (Lonza, Portsmouth, NH, USA) and incubated at 37 °C, 5 % CO_2, ≥ 90% RH.

Cell metabolic activity testing

The cells were plated at a density of 2 x 10^4 viable cells per well in 96-well culture plates and cultured for 24 h in EMEM (ATCC, Manassas, VA, USA) to allow cell attachment. In order to measure the cells' metabolic activity, the cells were exposed to selected concentrations of ≥ 99.5% DMSO (Sigma Aldrich Chemie, Steinheim, Germany, EU) and cultured for 4 h, 24 h, 48 h and 72 h. Concentrations of DMSO were 0.1-5%. In separate experiment the cells were exposed to 0.05%, 0.2%, 1% and 5% ethanol for 4 h and 24 h for comparison. The MTT Colorimetric Cell Viability Kit IV (PromoKine, PromoCell, Heidelberg, Germany, EU) was performed, following the manufacturer's instructions. Absorbance was measured spectrophotometrically at 570 nm (background absorbance at 630 nm) in pentaplicates for all samples. The percentage of the cells' metabolic activity was calculated with the following equation: (A_{570}-A_{630}) test sample value / (A_{570}-A_{630}) control value x 100, where A represents average value calculated from pentaplicates.

Cell morphology

Cell morphology was observed with an inverted microscope (DMI6000B, Leica) using a digital camera

(DFC365 FX Leica, Buffalo Grove, IL, USA).

Statistical analysis

Influence of different DMSO and ethanol concentrations on cells metabolic activity was determined by comparing metabolic activity of untreated cells (control). Kruskal-Wallis nonparametric test was used for statistical analysis, conducted using SPSS 25.0. Results are presented as mean ± SEM. The level of significance was set at $p < 0.001$.

Results and Discussion

Our results are the first to report the influence of DMSO on WM-266-4 cell line. Namely, after extensive literature review, none have investigated the effects of DMSO on melanoma cell line WM-266-4 yet. Inhibitory effect of DMSO on WM-266-4 cells' metabolic activity was observed, which is comparable with the results on other cell types (2, 4-6). After 4h, the inhibitory effect was significantly lower compared to longer times of exposure. Treating the cells with DMSO concentrations up to 1% resulted in approximately 40% inhibition of the cells' metabolic activity after 24h, 48h and 72h. Over 55% of WM-266-4 cells' metabolic activity was observed at 1.5% DMSO after 24h and 48h. When cells were exposed to 5% ethanol for 24 h approximately 20% inhibition of the cells' metabolic activity was observed. Ethanol had significantly lower effect on WM-266-4 cells' metabolic activity ($p < 0.001$) comparing to DMSO after 4 h and 24 h incubation. Nevertheless, high volatility of ethanol disables its use in experiments with longer incubation times and demands specific experimental setup to preserve the desired % of ethanol.

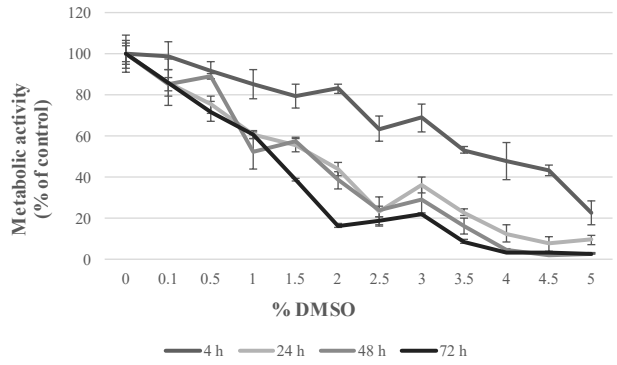

Figure 1. Influence of 0.1-5% DMSO on WM-266-4 cells' metabolic activity.

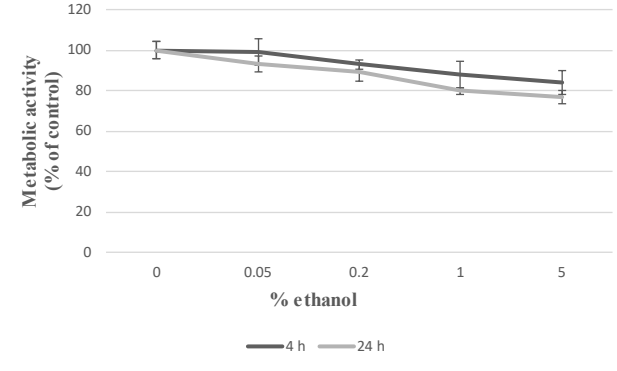

Figure 2. Influence of 0.05-5% ethanol on WM-266-4 cells' metabolic activity.

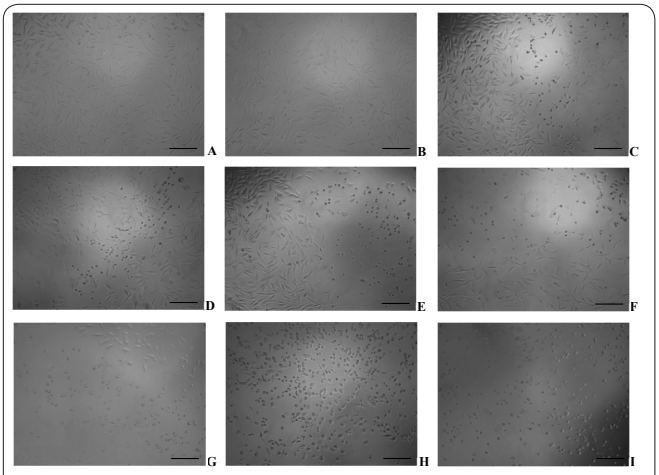

Figure 3. Morphological changes of WM-266-4 cells after 24 h exposure to DMSO: (**A)** control; (**B)** 0.1% DMSO; (**C)** 0.5% DMSO; (**D)** 1% DMSO; (**E)** 1.5% DMSO; (**F)** 2% DMSO; (**G)** 3% DMSO; (**H)** 4% DMSO and **(I)** 5% DMSO. Cells became round in shape. Scale bar, 100 μm.

Detailed results are presented in Figure 1 and Figure 2.

Furthermore, DMSO may have detrimental effects on cell morphology (13). In our study, morphological changes of WM-266-4 cells exposed to 1-5% DMSO were observed. Morphology of the cells changed progressively with the time of exposure (Figure 3). As shown by formed formazan granules in Figure 4, some of morphologically changed cells maintain their metabolic activity. Ethanol on the other side was shown to have no effects on cell morphology after 4 h and 24 h. Nevertheless, further investigations are needed to identify signaling pathways as well as to clarify cellular and molecular processes.

In summary, inhibitory effect of DMSO on WM-266-4 cells' metabolic activity was confirmed and the cells' morphological changes were observed. When testing highly insoluble substances even in DMSO on WM-266-4 cell line, concentrations of the solvent up to 1.5% could be used for *in vitro* experiments that not exceed 48h. However, DMSO should be used at the lowest possible concentration in biological assays.

Conflict of interest

The authors declare no conflict of interest.

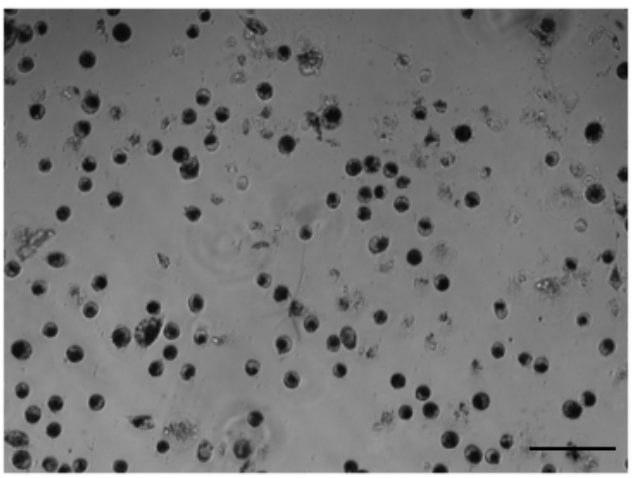

Figure 4. WM-266-4 cells with MTT formazan granules (1% DMSO, 24h exposure time). Despite morphological changes, certain cells form formazan granules. Scale bar, 50 μm.

Author's contribution

Both authors contribute equally to this manuscript.

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