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APPLICATION NOTE – JAGIELLONIAN CENTER OF INNOVATION

THE STUDY OF THE INFLUENCE OF DMSO ON HUMAN FIBROBLASTS PROLIFERATION IN-VITRO.

Katarzyna Pocheć[1], Tomasz Wójcik[1]

1. Jagiellonian Center of Innovation, ul. Bobrzyńskiego 14, 30-348 Kraków

ABSTRACT

Dimethyl sulfoxide is a commonly exploited solvent for water-insoluble chemicals and is widely used as solvent for different compounds in various cellular bioassays. Therefore, such experiments require evaluation of safe solvent concentration. This study shows that high concentrations of DMSO (2%-20%) lead to noticeable decrease in cell viability, whereas lower concentrations (0.05%-1%) show no significant difference.

INTRODUCTION

Dimethyl sulfoxide [(CH3)2SO; DMSO] is a clear, organic liquid with freezing point at 18.5°C. Polar, aprotic domain of the molecule results in its high affinity to water, while the apolar groups are responsible for the hydrophobic character. Due to its amphipathic nature, DMSO is remarkably miscible and therefore soluble both in aqueous and organic media. Hence, it is widely used as solvent for a variety of compounds, especially for water-insoluble molecules, and as a vehicle for drug therapy[1,2]. Given the fact that the effect of biological compounds is initially investigated mostly in cell cultures, solvent should be compatible with the growth media and should not affect cell viability. Therefore, it is extremely important to determine safe solvent concentration. Compound toxicity is commonly tested in vitro in fibroblast cultures [3,4l]. Toxic concentration of DMSO depends on the cell type, however OECD's (Organization for Economic Cooperation and Development) TG 487 guidelines recommend that organic solvents (including DMSO) should not exceed the concentration of 1% [5]. Solutions containing >1% DMSO have been reported as toxic for most mammalian cell types in in vitro culture assays[4].

The aim of this study was to analyze the viability of human fibroblast cells cultured in vitro in the presence of various DMSO concentrations, and to determine the safe concentration of DMSO.

RESULTS AND CONCLUSIONS

Shortly after 5 minutes of incubation with DMSO, significant decrease in cell viability can be observed: 42%, 40%, and 21%, respectively for DMSO concentrations of 5%, 10%, 20% (Figure 1). Viability keeps decreasing (Figures 2-5) and after 48 hours, it reaches the level of 1% for the highest DMSO concentration (Figure 6).

For 2% DMSO (Figures 1-5), the viability decrease was not so significant but still noticeable (viability range between 47% and 67%), which means that high concentrations of DMSO, >2%, cause cytotoxic effect. Lower DMSO concentrations (below 1%) didn't induce meaningful cell loss as compared to >2% DMSO: 70%, 74%, 74%, respectively, for 0,1%; 0,2%; 1% DMSO (Figure 1). As shown in Figures 1-5, changes in cell viability over time differ only slightly, which means that it is not possible to unambiguously determine the safest concentration, however these results are in compliance with OECD's guidelines. Therefore, 1% DMSO seems to be the critical concentration.

What is interesting, an increased number of living cells was observed, when the cells were cultured for 48 hours at the presence of lower DMSO concentrations (below 1%) (Figure 6). It is possible that low concentration of DMSO can lead to cell growth stimulation.

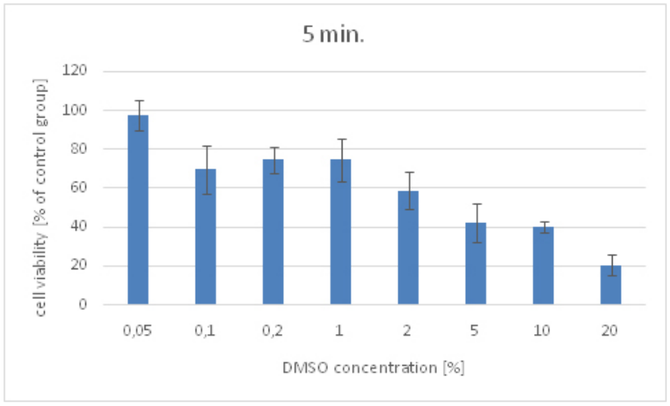

Figure 1. *Cell viability after 5-minute treatment with different DMSO concentrations.*

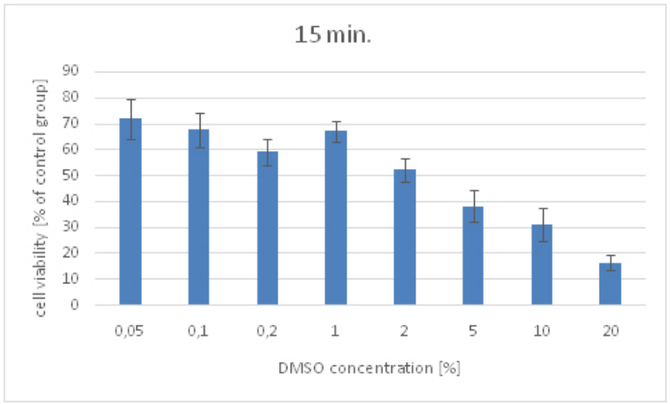

Figure 2. *Cell viability after 15-minute treatment with different DMSO concentrations.*

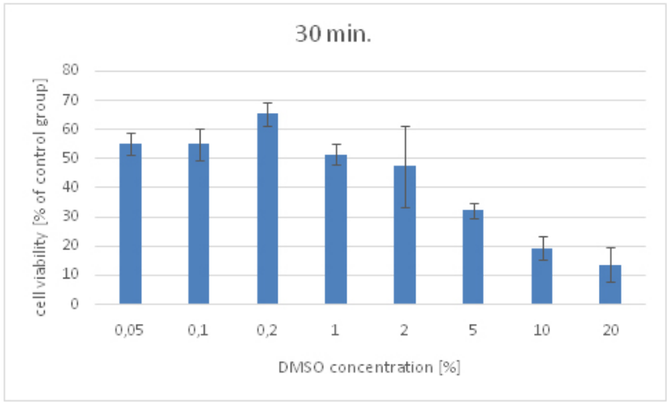

Figure 3. *Cell viability after 30-minute treatment with different DMSO concentrations.*

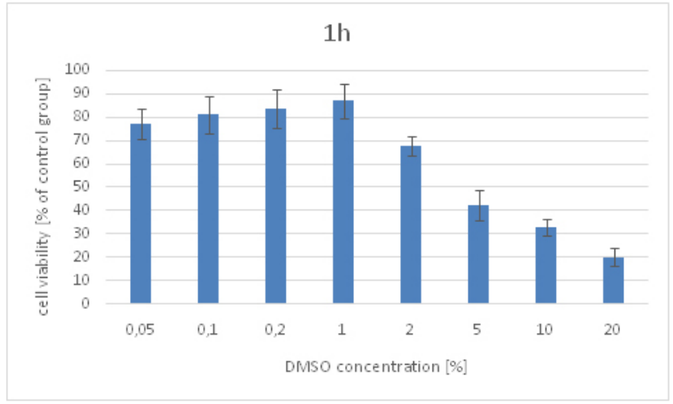

Figure 4. *Cell viability after 1-hour treatment with different DMSO concentrations.*

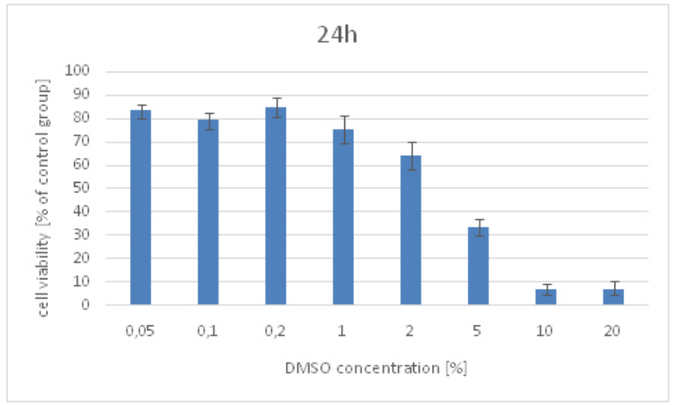

Figure 5. *Cell viability after 24-hour treatment with different DMSO concentrations.*

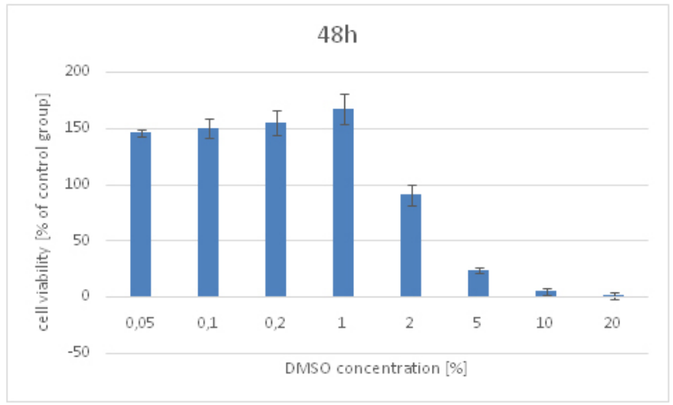

Figure 6. *Cell viability after 48-hour treatment hours with different DMSO concentrations.*

MATERIAL AND METHODS

CELL CULTURE

For this study, human dermal fibroblast cell line (HDFn line) was acquired from the ATCC Collection (ATCC® CRL-2522™).

The cells were initially cultured in humidified incubator at 37°C under 5% CO2 and 95% air atmosphere for 10 days. The growth medium consisted of Minimum Essential Medium (EMEM, LONZA), supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich) and 1% antibiotic solution (penicillin- streptomycin, Sigma-Aldrich).

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DMSO TREATMENT

After obtaining a sufficient number of cells, fibroblasts were seeded into six 96-well culture plates at a density of $5x10^3$ cells/well in triplicates and treated with 0,05%; 0.1.; 0,2%; 1%; 2%; 5%; 10%; 20% DMSO for the following periods of time: t_1= 5 min, t_2= 15 min, t_3= 30 min, t_4= 1h, t_5= 24 h, t_6= 48 h. The control group was represented by DMSO-free culture medium.

CELL VIABILITY

To evaluate cell viability, MTS test was performed. 20µL of MTS (CellTiter 96 AQueous One Solution Cell Proliferation Assay System; Promega) was put in contact with each well and incubated for 4 hours. Then, absorbance at 490 nm was measured with plate reader (TecanSpark 10M).

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CONTACT

Łukasz Kutrzeba, PhD
Director business development
K: +48 515 075 500
E: lukasz.kutrzeba@jci.pl

Jagiellonian Center of Innovation
ul. Bobrzyńskiego 14, 30-348 Kraków

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