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APPLICATION NOTE – JAGIELLONIAN CENTER OF INNOVATION

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

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ABSTRACT

Dimethyl sulfoxide is commonly exploited solvent for water insoluble chemicals and widely used as a solvent for the compounds in variety cellular based bioassays. Therefore these experiments require evaluation of the safe solvent concentration. This study presents that the high concentrations of DMSO (2%-20%) lead to noticeable decrease of cell viability, whereas lower concentrations (0,05%-1%) show no significant difference.

INTRODUCTION

Dimethyl sulfoxide [$(CH_3)_2SO$; DMSO] is a clear, organic liquid with freezing point at 18.5°C. The polar, aprotic domain of the molecule is the cause of its high affinity to water, while the apolar groups are responsible for the hydrophobic character. Due to this amphipathic nature of the molecule, DMSO is remarkably miscible and therefore soluble both in aqueous and organic media. Hence it is widely used as a solvent for variety of compounds, especially for the water insoluble molecules and as a vehicle for drug therapy [1,2]. Given the fact, that the effect of the biological compounds is predominantly, initially investigated on cell culture, the solvent should be compatible with the growth media and should not affect the cells viability. Therefore it is extremely important to evaluate the safe solvent concentration and culturing fibroblasts is a commonly used *in vitro* method for compounds toxicity testing [3,4l]. The 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 (which includes DMSO) should not exceed the concentration of 1% [5]. Solutions >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 analyse the viability of human fibroblast cells cultured *in vitro* at the presence of various DMSO concentration and to determine the safe concentration of DMSO.

RESULTS AND CONCLUSIONS

Shortly after 5 minutes of incubation with the DMSO it is possible to observe significant decrease of cell viability: 42%, 40%, 21% respectively for the concentrations 5%, 10%, 20% (Figure 1.). The viability keeps on decreasing (Figures 2-5) and after 48 hours it reaches the level of 1% for the highest DMSO concentration (Figure 6.).

For the 2% DMSO (Figures 1-5) the viability decrease was not so significant but still noticeable (viability range between 47% and 67%), which means that the high concentrations of DMSO, >2% causes cytotoxic effect. Lower concentrations of DMSO (below 1%) didn't lead to meaningful cell loss with comparison to the >2% DMSO. 70%, 74%, 74% respectively for the concentrations 0,1%; 0,2%; 1% (Figure 1.). As demonstrated in Figures 1-5, changes in cell viability in time differ but very slightly, meaning 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 a critical concentration.

Interesting is the fact that an increased number of live 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.

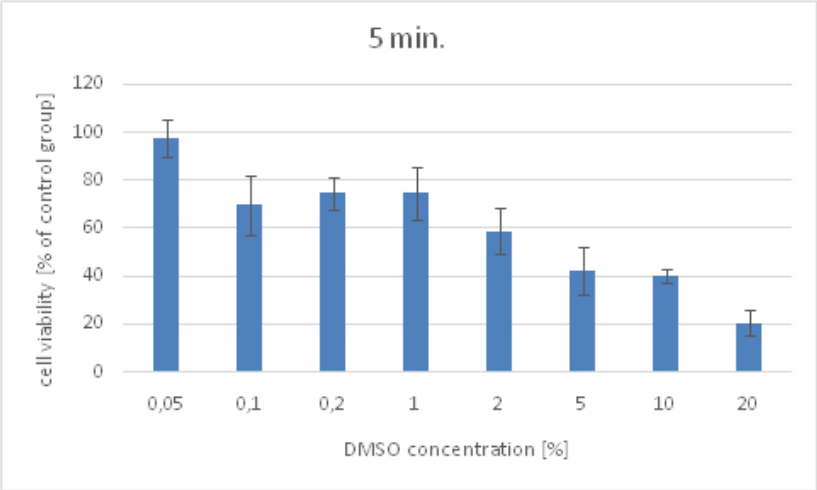

Figure 1. *Cell viability after treatment for 5 minutes with different DMSO concentrations.*

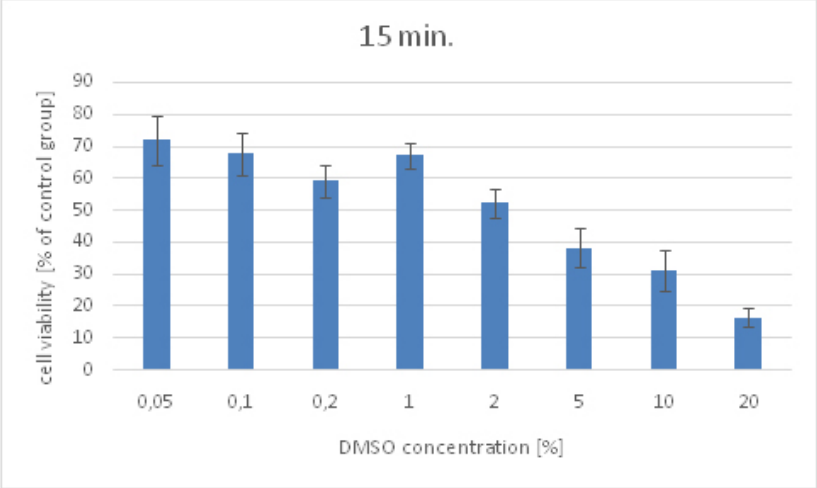

Figure 2. *Cell viability after treatment for 15 minutes with different DMSO concentrations.*

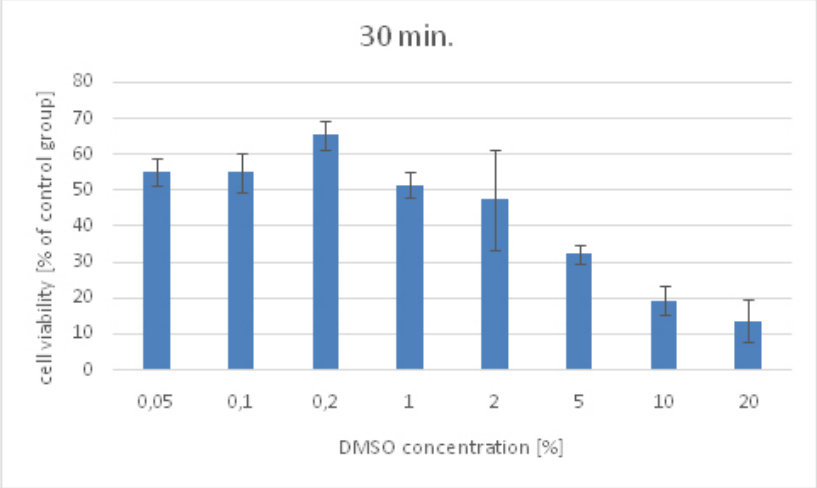

Figure 3. *Cell viability after treatment for 30 minutes with different DMSO concentrations.*

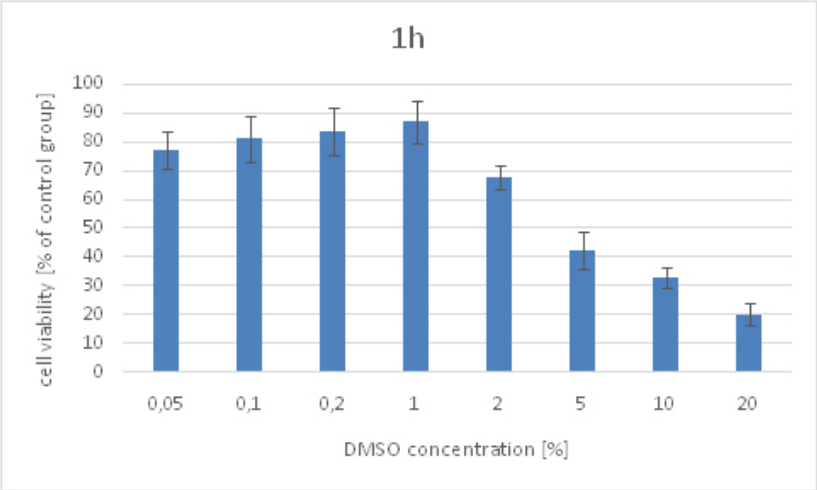

Figure 4. *Cell viability after treatment for 1 hour with different DMSO concentrations.*

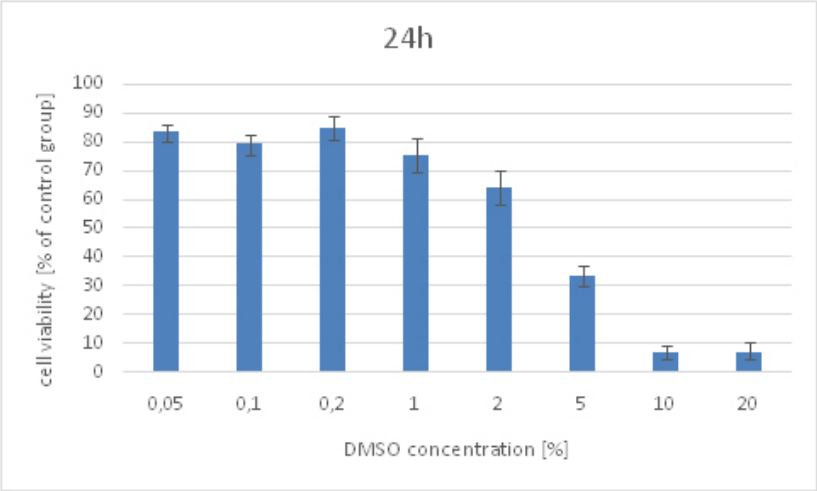

Figure 5. *Cell viability after treatment for 24 hours with different DMSO concentrations.*

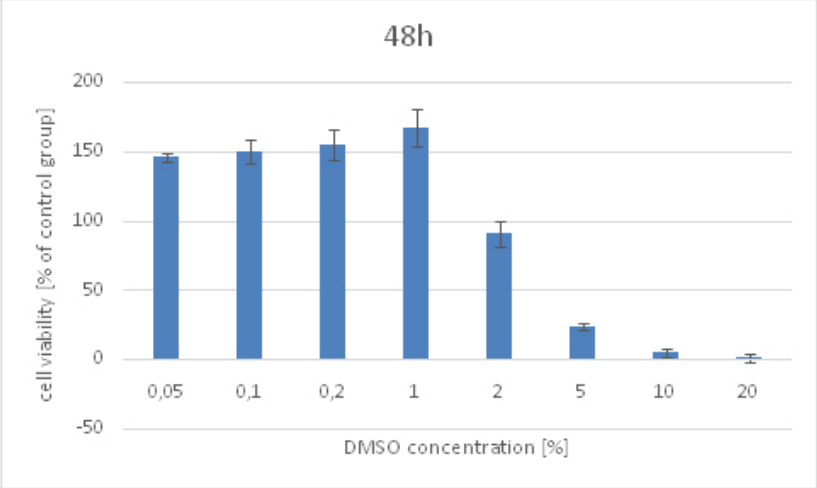

Figure 6. *Cell viability after treatment for 48 hours with different DMSO concentrations.*

MATERIAL AND METHODS

CELL CULTURE

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

The cells were initial cultured for 10 days in humidified incubator at 37°C under a 5% CO_2 and 95% air atmosphere. The growth medium consisted of Minimum Essential Medium (EMEM, LONZA), supplemented with 10% foetal bovine serum (FBS, Sigma-Aldrich) and 1% of antibiotics solution (penicillin- streptomycin, Sigma-Aldrich).

DMSO TREATMENT

After obtaining the 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% of the DMSO for the following periods of time: t_1= 5min, t_2= 15 min, t_3= 30 min, t_4= 1h, t_5= 24h, t_6= 48h. The control group was represented by DMSO-free culture medium.

CELL VIABILITY

To evaluate the cells viability the 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 the absorbance at 490 nm was measured with the plate reader (TecanSpark 10M).

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