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RADIO-PROTECTIVE EFFECT OF DMSO AND GLYCEROL IN HUMAN NON-SMALL CELL LUNG CANCER IRRADIATED WITH GAMMA RAYS

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ABSTRACT
Direct effects of radiation affect the DNA molecule, causing DNA damage and finally cell death. We examined the role of DMSO and glycerol as free-radical scavengers in HTB177 cells irradiated with gamma rays. Direct effects of radiation were estimated through DNA double strand break (DSB) quantification and cell survival. Results of this work revealed that chosen concentration of DMSO exhibit higher protective effect comparing to glycerol.

INTRODUCTION
It is known that ionizing radiation causes cell damage through direct and indirect effects [1]. Direct effects refer to straight interaction of gamma rays or charged particles with DNA molecule causing damages (single and double strand breaks, modification of bases and DNA backbone). If not repaired properly, DNA lesions provoke genomic instability and consequently lead to cell death. Indirect effects of radiation refer to formation of free radical species during water hydrolysis. These highly reactive ions interact with DNA, but also with other biomolecules and cellular compartments, triggering oxidative stress [1]. In the field of radioprotection, many chemical compounds are tested for their capacity to prevent DNA and cell damage. It is known that DMSO and glycerol act as free radical scavengersthus lowering oxidative stress caused by indirect effects of radiation.In this study we used non-toxic concentrations of DMSO and glycerol that represent low and high scavenging conditions (1 and 100mM DMSOand 2 and 200 mM glycerol)[2, 3].

Clonogenic assay is used in radiobiology as standard test to determine cell survival after irradiation alone or in combination with chemical

treatment [4]. The impact of DMSO and glycerol on radio-sensitivity of HTB177 non-small lung cancer was tested.

Phosphorylated histone H2AX (γH2AX) acts as a sensor of DSB. Due to its role in DNA repair, it is used as a DSB biomarker. Immunocytochemical analysis of γH2AXisconsidered to be sensitive method for DSB detection and quantification [5].

EXPERIMENTAL

The HTB177 cells were purchased from the ATCC (Rockville, MD, USA) and grown as a monolayer culture in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in humidified atmosphere at 5% CO_2 and 37°C (Heraeus, Hanau, Germany).

Cells were grown in 3 ml of serum-free medium for 18 h and seeded in six well culture dishes for clonogenic assay and on cover slips for immunocytochemical analyses. One hour prior to irradiation, scavengers were added in final concentrations of 1 and 100mM (DMSO) and 2 and 200 mM (glycerol).The medium was discarded andcells were irradiated with gamma rays from ^{60}Co source at the dose rate of ~ 1 Gy/min. Irradiation was performed in air at room temperature at the Vinča Institute of Nuclear Sciences, Belgrade, Serbia. After irradiation, cells were incubated in medium without DMSO and glycerol.

Irradiation doses for clonogenic assay were 0.5, 1, 2, 3, 4, 5 Gy. After seven days, cells were fixed and stained with 10% Giemsa solution [4]. More than 50 cells per colony were scored as a surviving cell. Survival was calculated by comparing the number of colonies in irradiated dishes with untreated control. Dose dependent cell survival data were fitted using the linear-quadratic equation: $S = \exp(-\alpha D - \beta D^2)$, where S is the surviving fraction for the dose D, while α and β are the fitting parameters. Surviving fractions were evaluated from the best fit survival curves using the computer software TableCurve 2Dv 5.00.

Immunocytochemical analyses were performed as previously described [4]. Cells were fixed 30 minutes after irradiation with the dose of 1 Gy. Fluorescently labeled phosphorylated H2AX antibody (Alexa Fluor 488, BioLegend Inc. San Diego, California, United States) was used. Micrographs were made on laser confocal microscope Leica TCS SP5 II in LAS AF Lite software program (Leica Microsystem CMS GmbH). γH2AX foci were quantified using Image J software.

Statistical analyses of experimental results were done using Student's *t* test and the level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Comparing to irradiated control, SF2 value was significantly higher in cells pre-treated with 100 mM DMSO($p<0.05$), while 1 mM DMSO had no significant effect on SF2 (Figure 1a). Therefore, 100 mM DMSO expressed higher radio-protective effect after seven days of incubation. Lower concentration of glycerol slightly stimulated cell survival while higher decreased SF2 but with no statistical significance (Figure 1.b). These results indicate that 2 mM glycerol may have radio-protective and 200 mM glycerol cytotoxic effect seven days after the treatment.

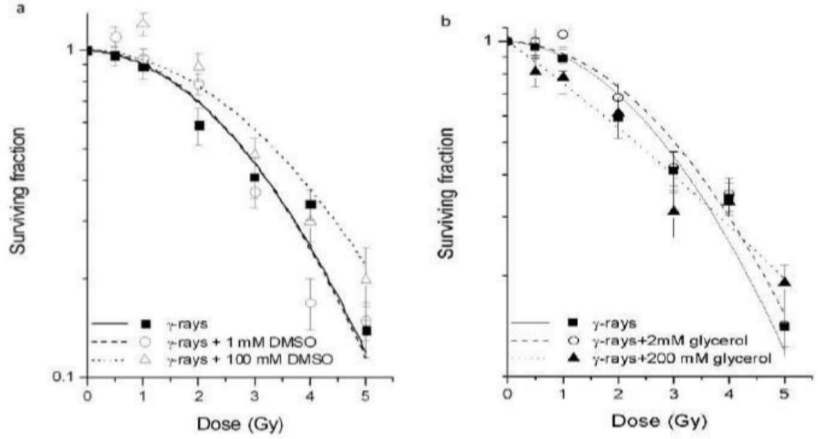

Figure 1. Clonogenic survival of HTB 177 cells 7 days after irradiation with 0.5, 1, 2, 3, 4, 5 Gy of gamma rays. Cells were treated with 1 and 100 mM DMSO (a) or 2 and 200 mM glycerol (b). Results are shown as mean±SEM.

DNA DSBs were analysed through the formation of γH2AX foci 30 minutes after irradiation, when the level of γH2AX expression was the highest [5]. Dose of 1 Gy was selected for fine detection of foci, as higher doses could cause signal overlapping [6]. The number of foci per cell were significantly lower in both experimental groups treated with DMSO comparing to irradiated control ($p<0.001$) (Figure 2.a) implicating its radio-protective role. 200 mM glycerol decreased number of foci per cell, ($p<0.001$) (Figure 2.b) thus showingradio-protective effect.

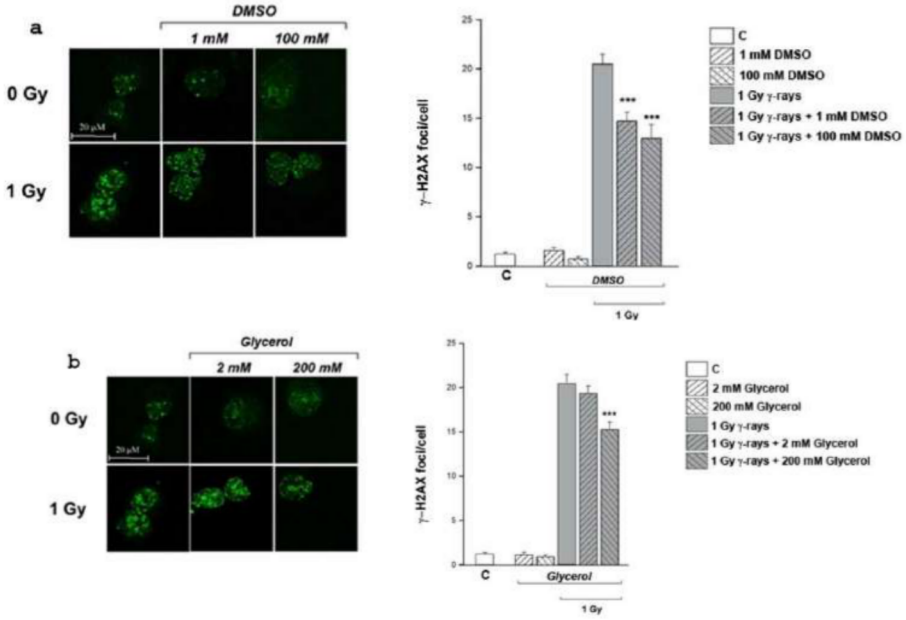

Figure 2. Immunocytochemical analyses of γH2AX in DMSO (a) and glycerol (b) treated cells irradiated with 1 Gy of gamma rays. Results are shown as mean value of γH2AX foci per cell ±SEM.

CONCLUSION

The results showed that DMSO and glycerol pre-treatment can stimulate cell survival seven days after irradiation with gamma rays. The possible mechanism is inhibition of DNA lesions by reducing the level of DNA DSBs. These data will be further analysed.

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