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Cytotoxic effect of dimethyl sulfoxide (DMSO) on hematopoietic stem cells: Influence of the temperature and the incubation time

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The cryoprotection is crucial for the preservation of haematopoietic stem cells, which allows postponing of their transplantation after the isolation, without significant decrease of the cell viability even after years. Dimethyl sulfoxide (DMSO) is a widely used intracellular cryoprotectant. Its small amphiphilic molecule fast penetrates through the cytoplasmic membrane. It is known that DMSO is toxic above 4°C but its effect on the hematopoietic stem cells is insufficiently studied. The purpose of this investigation is to analyze the influences of the duration and the temperature of incubation in a 5 % solution of DMSO in saline on the viability of the hematopoietic stem cells, using trypan-blue exclusion test. The results show out that at 4°C DMSO has a low cytotoxic effect even after 24 hours of incubation. At room temperature the viability decreases by 67% for the same time of treatment; and at 37°C the 24-hour incubation leads to 100% cell mortality.

Keywords: DMSO cytotoxicity, cryoprotection, stem cells

INTRODUCTION

Hematopoietic stem cell (HSC) transplantation is widely used in the therapy of benign and malignant hematological diseases and the number of autologous and allogeneic transplantations rises every year [1-3]. The hematopoietic stem cells from bone marrow, peripheral blood or cord blood are with intensive metabolism, therefore, their storage time at a temperature between +2 and +24°C is limited [4, 5]. The storage of stem cells between +4ºC and +8ºC is accepted as safe for up to 5 days, after which their viability drops markedly [6-8], so their longer storage until the day of transplantation is possible only in a frozen state. The cryoprotection is crucial for the freezing of living cells, as intracellular cryoprotectants should penetrate the cell membrane not allowing dehydration that causes osmotic injury [9]. Dimethyl sulfoxide (DMSO) is a widely used intracellular cryoprotectant [10] for haematopoietic stem cells. Its small amphiphilic molecule quickly penetrates through the cytoplasmic membrane. Despite slower passage through cell membrane in comparison with water [11], the membrane permeability P of DMSO is 0.157 μm/s for model membranes [12] and 0.13 μm/s for red blood cells [13], which is approximately three times faster than this of glycerol. The presence of negatively charged atoms like sulfur and oxygen, determines the dipole and nucleophilic character of its molecule, thus making DMSO proton acceptor in hydrogen bonds generation. The hydrogen bonds, generated between DMSO and two water molecules, are 1.3 times stronger than the hydrogen bonds between water molecules themselves. Resulting from this, DMSO disturbs the water molecules organization and impedes ice formation inside the cell during freezing [14]. Additionally, the use of this intracellular cryoprotectant leads to widening of the temperature diapason of the dehydration, so the cell has more time for response to changes in osmotic pressure [15].

A major requirement for the cryoprotectants, in addition to their protective role during cell freezing, is to be nontoxic for the cells at high molar concentration necessary for prevention of extreme ice formation [9]. The cytotoxicity of DMSO depends on its concentration [13, 16, 17] and the type of the treated cells. It is known that DMSO is toxic above 4°C but its effect on the hematopoietic stem cells is insufficiently studied. The temperature of incubation, ranging from 4°C to 37°C at low DMSO concentration for short periods does not affect cytotoxicity. For instance, neonatal human dermal fibroblasts retain their viability after 30-min incubation in 5% DMSO at temperatures of 4°C, 25°C and 37°C [18].

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Similar effect is observed in human chondrocytes, where the incubation at temperatures of 4°C, 22°C and 37°C does not change the cell viability with 1M (approximately 8%) DMSO for 120 min [19]. Low dose cytotoxicity is observed in retinal ganglion cells after 24-hour incubation with 4% DMSO [20].

Despite the fact that DMSO is a widely used intracellular cryoprotectant for different animal cells, including stem cells, the effect of incubation temperature on DMSO cytotoxicity after longer incubation periods is not explored in detail. The purpose of our work is to study the correlation between incubation temperature and cytotoxicity on human hematopoietic stem cells after exposure to 5% DMSO for up to 24 hours.

MATERIALS AND METHODS

Hematopoietic stem cells from peripheral blood of stimulated donors and patients were collected using a cell separator. The cell suspension was concentrated by removing the plasma. A cryoprotective solution was added so that the final concentration of DMSO was 5%; of hydroxyethyl starch (HES) 450 kg/mol 3.6% and of human serum albumin (HSA) 3%. The viability of the stem cells mixed with cryoprotective solution, was tested by trypan-blue exclusion after incubation at 4°C, room temperature and 37°C for 30 min, 1 hour and 24 hours. Trypan-blue test is based on the different membrane permeability of living and dead cells for dyes. Trypan blue is a large, hydrophilic, tetrasulfonated anionic dye. The intact lipid bilayer of the cell membrane is an impermeable barrier for this relatively large and negatively charged molecule. Thus, the living cells remain uncolored, while the dye penetrates in the dead cells and they are colored in intensive blue.

RESULTS

At the lowest temperature, the number of dead cells (colored in blue) practically does not change, while at higher temperatures their number significantly increases with the incubation time. On Figure 1 are presented microscopic pictures of HSC suspension incubated with 5% DMSO at different temperatures and stained with trypan blue after 30, 60 min and 24 hours (Fig. 1A at 4°C, Fig. 1B at 20°C, and Fig. 1C at 37°C).

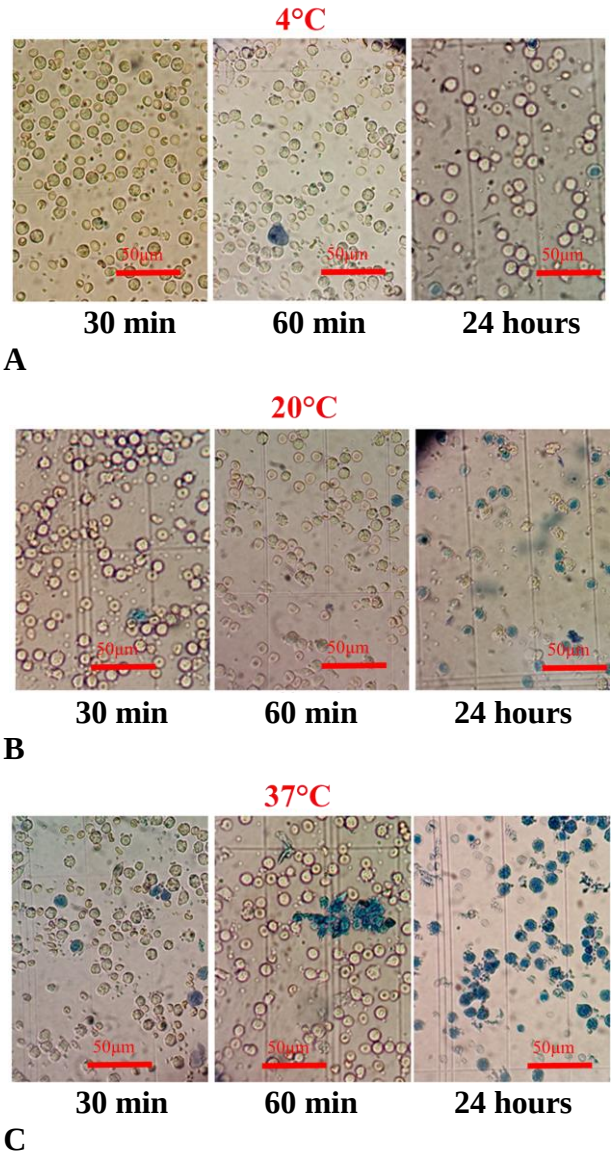
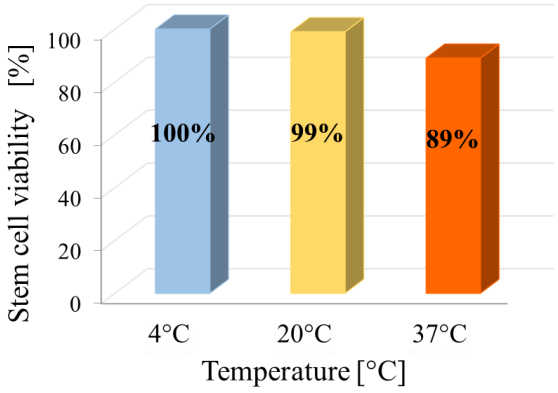


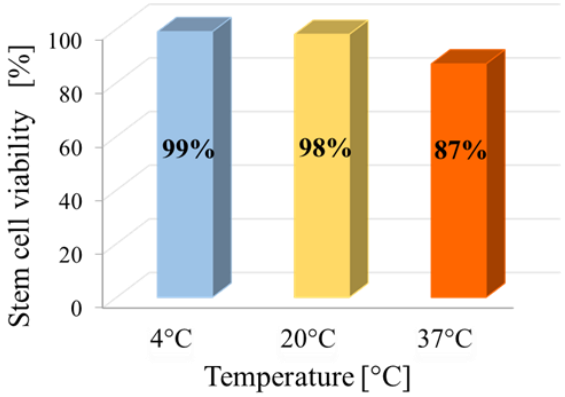
Fig. 1. Microscopic pictures of trypan-blue test for stem cell viability at 4°C (A), 20°C (B) 37°C (C) temperature of incubation. The dead cells are colored in blue.

The percentage of viable stem cells, shown after incubation at different temperatures with a standard cryoprotective solution, used for hematopoietic stem cell freezing, can be seen on Figure 2. The viability of the stem cells, when incubation periods are short, is much less affected by DMSO and its cytotoxic action is practically seen only at 37°C. One-hour incubation of peripheral blood stem cells leads to insignificant viability changes in comparison with 30-min incubation (Fig. 2B).

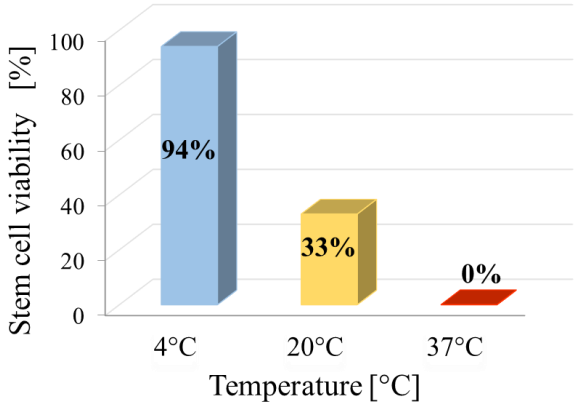
The influence of the temperature on the cytotoxic action of DMSO becomes apparent on the 24th hour of incubation of the stem cells with standard cryoprotective solution (Fig. 2C). At 4 °C the viability still remains very high – 99% and DMSO cytotoxicity is practically not seen. At room temperature the one-day incubation decreases the cell viability to 33%, and incubation at 37 °C leads to complete loss of cell viability.



A



B



C

Fig. 2. Stem cell viability after 30 min (A), 60 min (B) and 24 hours (C) of incubation in cryoprotective solution with 5% DMSO. The standard deviation did not exceed 5%.

The influence of incubation temperature on DMSO cytotoxicity on the 24th hour is so apparent, that the changes in the hematopoietic stem cell suspension in standard cryoprotective solution can be seen macroscopically by the change of its color (Fig. 3). We suppose that this macroscopic change in the coloration of the sample results from the connection of the hem ring of the red blood cells which are part of the cell suspension, and the change in its electronic structure, changing its absorption spectrum.

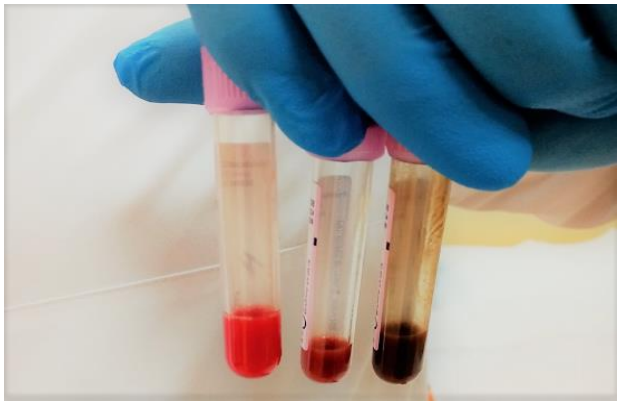


Fig. 3. Macroscopic picture of stem cell suspension after 24 hours of incubation in cryoprotective solution. The cells, incubated at 37ºC, are with darkest color.

DISCUSSION

DMSO causes major alterations in structure and function of mammalian cell membranes, proteins and nucleic acids. Membrane lipids are subjected to peroxide oxidation with injury resulting in mitochondrial membrane potential reduction, proteins are denaturated and the structure of the nucleic acids is changed. Time of incubation and DMSO concentration are important factors. Our results show that the temperature of exposure is also important for DMSO cytotoxicity. Exposure of cells to DMSO at low concentation (less than 10%) even at 37°C for 1 hour does not result in a gross loss of viability. For practical purposes, this would imply that addition of cryoprotectants that have low DMSO concentration can be performed sucessfully at ambient (22°C) room temperature.

CONCLUSIONS

Five percent DMSO is toxic for hematopoietic stem cells after long periods (more than 1 hour) and high (above 22°C) temperature of incubation. After 24 hours of incubation at room temperature, the viability decreases much faster and at 37°C 100 % cell loss is registered. The cell viability decreases after 1 hour only at a temperature higher than 20°C. DMSO cytotoxicity is low even after 24 hours of incubation. Currently, cryoprotectants are cooled to

4°C prior to addition to the cell suspension and the cells are frozen as soon as possible. Our results show that manipulation of cells (addition of cryoprotectant solution) at ambient temperature for a short period of time (up to 60 min) is also safe and will not result in additional loss of viability.

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