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Review

Hydroxyethylstarch in cryopreservation – Mechanisms, benefits and problems

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ABSTRACT

As the progress of regenerative medicine places ever greater attention on cryopreservation of (stem) cells, tried and tested cryopreservation solutions deserve a second look. This article discusses the use of hydroxyethyl starch (HES) as a cryoprotectant. Charting carefully the recorded uses of HES as a cryoprotectant, in parallel to its further clinical use, indicates that some HES subtypes are a useful supplement to dimethysulfoxide (DMSO) in cryopreservation. However, we suggest that the most common admixture ratio of HES and DMSO in cryoprotectant solutions has been established by historical happenstance and requires further investigation and optimization.

Contents

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1. Introduction

Demands for improved cell, tissue and organ storage are increasing as more and more products of regenerative medicine reach the clinic. The safety of cryopreservation of cell material is one of many emerging considerations in regenerative medicine. In addition use of cell-based assays for drug screening and safety testing raises questions as to what cryopreservation methods are preferable.

After briefly charting considerations in cryopreservation and cryoprotection, we will focus on one cryoprotectant factor in particular: We will consider the capacity of hydroxylethyl starch (HES) to act as a cryoprotectant, its use and its reported efficacy from the available data (which, although reaching back 40 years is still rather sparse) and consider the safety implications of using HES- in particular in comparison with the most common cryoprotectant compound dimethyl sulfoxide (DMSO).

2. Cryoprotection basics

Upon cooling with cryoprotectants, extra- and intracellular viscosities abruptly increase whereas thermal energy is decreased and not sufficient to enable chemical reactions. In this case all biological reactions are slowed down to a minimum that makes long term storage of cells, tissues and organs possible. The damages induced by cryopreservation involve many different cell compartments but the exact mechanisms are surprisingly poorly understood. In general, a distinction can be made between the effects of ice formation and other stresses.

2.1. Ice crystal formation

The high concentration of H_2O in tissues or cells is one of the leading determinants of the physical changes during the cooling and warming process. During phase transition of the aqueous solutions, ice crystal formation occurs, and can lead to great damage to tissues and cells [1]. Membrane-associated cell damage can vary greatly between different species, depending on the membrane composition [2]. However, there are doubts if cell membranes rupture [3] due to ice formation.

Ice crystal formation in water solution can occur at any time and any extend at temperatures below 0 °C [1]. The ice is normally solid and has a regular crystalline structure. Intracellular water can remain in a super cooled unfrozen state, even at temperature between −5 °C and −40 °C. [4,5].

Ice crystals need to have a starting point, a nucleus, from where they are able to grow. Examples for nuclei are: ions, vibration and ice crystals themselves. Ice can also form in the extracellular space leading to increasing concentrations of electrolytes in the remaining extracellular solution. The growing extracellular ice forms channels where the extracellular solution and the cells are displaced [6]. In these channels the resulting pressure can cause lethal cell deformation [3]. The evidence that extracellular ice is harmful for tissues was found on cryopreserved smooth muscle tissue. When ice is forming extra-cellular only 21% recovery is observed, compared to 74% for unfrozen samples [7].

Ice growth can be transferred from one cell to another via gap junctions [8]. In addition, transmembrane proteins, "aquaporines", can initiate ice crystal growth from one side to the other side of the cell membrane [3].

Intracellular ice formation can therefore be induced by extracellular ice without damage of the cell membrane. The intracellular super cooled water tends to flow from the intra- to extracellular space due to its higher vapor pressure than ice. Due to the highly concentrated extracellular solution, the intracellular water diffuses to the outside (osmosis), resulting in cell dehydration [9]. The osmotically induced flow of water through the cell-membrane has also been proposed as a cause of damage [10]. As it is known that intracellular ice damages the cells mechanically [4], the outflow of water may in fact be a damage-reducing factor.

2.2. Other freezing-associated stresses

Cryopreservation can induce apoptosis or necrosis [11] which can be reduced by adding anti-apoptotic factors [11] or antioxidants [12]. One of the possible triggers for those types of damage might be an increase in reactive oxygen species (ROS), usually hydrogen peroxide (H_2O_2), superoxide anions, and hydroxyl radicals, production during cryopreservation [13–15]. There are data showing that human sperm DNA fragmentation is associated with an increase in oxidative stress during cryopreservation, rather than the activation of caspases and apoptosis [16]. Excessive and increased generation of ROS followed by peroxidation of membrane phospholipids are proposed as one of the biochemical basis of damaging effect during sperm cryopreservation [17]. Decreased glutathione levels found in frozen cells [18,19] as well as reduced antioxidative defense activities like SOD, catalase and others [20] will be involved in a net increase of ROS during cryopreservation. Cryopreserved retinal pigment epithelial cells showed increased expression of senescence-associated beta-gal activity, increased single-strand DNA breaks in telomeric regions and subsequently accelerated telomeric loss after thawing [21]. Cryopreservation also promotes DNA strand breaks in other regions and induces alterations in damage repair systems [22]. It was shown in human lymphocytes that cryopreservation lead to a decreased ability to repair DNA damage after hydrogen peroxide challenge [23]. One of the key DNA repair enzyme, H2AX histone protein, was found to be phosphorylated and activated in two different cell lines in response to freezing at −20 °C and −80 °C. There is a possibility that H2AX autophosphorylates at freezing temperature to preserve genetic integrity. But it is also possible that freezing cells induces disulfide bond formation through oxidative stress [24].

2.3. Cryoprotection

In order to improve the survival of cryopreseved cells, cryoprotectant agents (CPAs) are used. Major effects of CPAs are determined by their ability to reduce the freezing and thawing point and to lower the optimal cooling rate.