Cryobiology 68 (2014) 50–56

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

# Dose–injury relationships for cryoprotective agent injury to human chondrocytes ☆

M.D. Fahmy [a], K.A. Almansoori [a], L. Laouar [a], V. Prasad [b], L.E. McGann [c], J.A.W. Elliott [b,c], N.M. Jomha [a,*]

[a] Division of Orthopaedic Surgery, Department of Surgery, University of Alberta, Edmonton, AB, Canada
[b] Department of Chemical and Materials Engineering, University of Alberta, Edmonton, AB, Canada
[c] Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada

ARTICLE INFO

*Article history:*
Received 10 April 2013
Accepted 11 November 2013
Available online 20 November 2013

*Keywords:*
Human
Chondrocytes
Injury
Cryoprotective agents
Vitrification
Dose
Cryopreservation

ABSTRACT

Vitrification of articular cartilage (AC) could enhance tissue availability but requires high concentrations of cryoprotective agents (CPAs). This study investigated relative injuries caused by commonly used CPAs. We hypothesized that the *in situ* chondrocyte dose–injury relationships of five commonly used CPAs are nonlinear and that relative injuries could be determined by comparing cell death after exposure at increasing concentrations. Human AC samples were used from four patients undergoing total knee arthroplasty surgery. Seventy μm slices were exposed in a stepwise protocol to increasing concentrations of 5 CPAs (max = 8 M); dimethyl sulfoxide ($Me_2SO$), glycerol (Gly), propylene glycol (PG), ethylene glycol (EG), and formamide (FM). Chondrocyte viability was determined by membrane integrity stains. Statistical analysis included t-tests and nonlinear least squares estimation methods. The dose–injury to chondrocytes relationships for all CPAs were found to be nonlinear (sigmoidal best fit). For the particular loading protocol in this study, the data identified the following CPA concentrations at which chondrocyte recoveries statistically deviated significantly from the control recovery; 1 M for Gly, 4 M for FM and PG, 6 M for $Me_2SO$, and 7 M for EG. Comparison of individual means demonstrated that Gly exposure resulted in the lowest recovery, followed by PG, and then $Me_2SO$, FM and EG in no specific order. The information from this study provides an order of damage to human chondrocytes *in situ* of commonly used CPAs for vitrification of AC and identifies threshold CPA concentrations for a stepwise loading protocol at which chondrocyte recovery is significantly decreased. In general, Gly and PG were the most damaging while DMSO and EG were among the least damaging.

© 2013 Elsevier Inc. All rights reserved.

## Introduction

Osteoarthritis is the leading cause of work disability in Canada, with an annual estimated economic productivity and long-term disability cost of $4.4 billion dollars [1,16]. When injured, articular cartilage (AC) has limited ability to repair itself and the injury may progress to disabling degenerative arthritis [36]. Due to the low immunogenicity and avascularity of cartilage tissue, osteochondral lesions can be treated with articular cartilage allograft transplantation [15]. However, in order to overcome transport, sizing, and availability challenges, an effective method for long-term preservation of these allografts must first be developed and optimized. Vitrification of AC is one method that can preserve this tissue indefinitely and involves the use of high concentrations of cryoprotective agents (CPAs) and rapid cooling rates [20,28,30]. One challenge is to limit the injury from the CPAs contained within the vitrification solution, while still obtaining an adequate tissue-concentration sufficient to vitrify.

Several factors affect CPA permeation into tissues including temperature, exposure time, solution agitation, and solution concentration [2,3,5,21,30,35]. However, a general limitation on vitrification of tissues such as AC has been the cellular injury of the CPA during the time required for tissue loading. Cryoprotective agents are categorized into either *permeating* (e.g., dimethyl sulfoxide, glycerol, ethylene glycol, formamide, etc.) or *non-permeating* compounds (e.g., sucrose, trehalose, etc.), depending on their ability to penetrate the cell membrane. Because permeating CPAs can penetrate into the cell, they are generally more effective for vitrification but are also more toxic, relative to non-permeating CPAs [24,30]. The mechanisms and severity of these toxicities vary among the CPAs and their tissue targets [22,30]. For example, glycerol (Gly) and ethylene glycol (EG) affect cellular metabolism; dimethyl

☆ *Statement of funding:* The research was funded by the Canadian Institutes for Health Research (MOP 93805) and the Edmonton Orthopaedic Research Committee. J.A.W.E. holds a Canada Research Chair in Thermodynamics.

* Corresponding author. Address: 2D2.28 WMC, Department of Surgery University of Alberta Hospital, 8440-112St Edmonton, AB T6G 2B7, Canada. Fax: +1 780 407 2819.
*E-mail address:* njomha@ualberta.ca (N.M. Jomha).

0011-2240/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cryobiol.2013.11.006

sulphoxide ($Me_2SO$) disrupts cell membrane ion channels; formamide (FM) is damaging to cellular cytoskeletal architecture and function; and propylene glycol (PG), $Me_2SO$ and FM, have been shown to exhibit genotoxic effects [6,8,17–19,26]. In fact, not only do different CPAs exhibit different degrees of toxicity on cells of the same cellular lineage, but the degree and order of toxicity among the CPAs from the most to least toxic, can be different for the same cell type (e.g. chondrocytes) across different animal species [4,11,31,33–35].

As a result of the various mechanisms and complexities of CPA toxicity, the exact relationship between a CPA's dosing protocol (concentration and exposure time) and the resulting human chondrocyte viability is unknown. Dose–toxicity relationships are, in general, nonlinear [12]. We hypothesized that the *in situ* human chondrocyte dose–injury relationships for particular step-loading protocols are nonlinear and that the relative injuries of five commonly used CPAs could be determined by comparing cell death after exposure to increasing concentrations. To determine this, we exposed human chondrocytes *in situ* in AC slices to increasing concentrations of five commonly used permeating CPAs and measured the chondrocyte recovery. Cartilage slices with chondrocytes within their normal microenvironments (i.e., *in situ*) were used because a previous study demonstrated that cryopreservation characteristics of cells change when they are released from their natural extracellular matrix [30].

## Materials and methods

### *Cryoprotective agent solution preparation*

Five CPAs were used: dimethyl sulphoxide ($Me_2SO$), ethylene glycol (EG), propylene glycol (PG), glycerol (Gly) and formamide (FM) (Fisher Scientific Co., Ottawa, ON). Experimental solutions were prepared from 12 M (M) stock solutions by diluting them with sterile X-VIVO [Lonza Group Ltd, Basel, Switzerland] to 1, 2, 3, 4, 5, 6, 7, and 8 M concentrations. The 12 M stock solutions were prepared by adding the required mass of CPA to phosphate buffered saline to the required total solution volume. Experimental solutions were stored in a 4 °C fridge for use within 24 h of preparation. Each of the five CPAs were placed into a 96-well cell culture plate with increasing solution concentrations and labelled appropriately. This resulted in a total of 40 wells of increasing CPA concentrations at 1 M increments. In addition, 16 wells were prepared containing just X-VIVO for use in dilution steps at the end of the protocols. All wells contained 1 mL of liquid.

### *Cartilage tissue isolation*

After approval from the University of Alberta Research Ethics Board, a total of 4 AC samples were obtained from 4 donor patients undergoing elective total knee arthroplasty surgeries and transported in sterile phosphate-buffered saline [PBS, pH 7.1, Invitrogen, Carlsbad, CA]. Only AC samples graded as Outerbridge grade 0 or 1 were used [29,32]. Full thickness osteochondral dowels of 10 mm diameter were cored and the AC was cut (perpendicular to the articular surface) into 70 μm thick slices using a vibrotome [The Vibratome Company, St. Louis, MO]. The slices were immersed in a petri-dish containing X-VIVO solution [Lonza Group Ltd, Basel, Switzerland] at room temperature, and used within 120 min of preparation. For each repeat of the experiment, sufficient numbers of slices were obtained from each donor to perform all treatment conditions as well as the positive and negative controls. Therefore, all four donors provided sufficient tissue to complete four independent repeats of the experiment.

### *Cryoprotective agent injury testing*

Once the CPA solution concentrations were loaded into the 96-well plates and an adequate number of cartilage slices were obtained, testing of the above mentioned five CPAs were undertaken at room temperature (~21 °C). Rather than directly placing the cartilage slices into the respective final CPA testing concentrations, the slices were exposed to the CPAs in sequential stepped 1 M increments to allow the cells time to equilibrate with their surrounding extracellular osmotic environments. This osmotic 'ladder' CPA exposure protocol is regularly used during vitrification protocols. Otherwise, directly placing a cartilage slice into a 6 M or 8 M CPA concentration solution can result in hyperosmotic-solution injury in addition to CPA-toxicity injury, and therefore confound our results. The ladder protocol involved immersing slices in a stepwise fashion beginning at 1 M CPA solution (1 ml) for 5 min, before transferring slices to the next incremental solution (i.e., 2 M) for another 5 min, and continuing until the CPA testing solution concentration was reached. Once a slice reached its intended testing concentration, it remained in that final testing concentration for a 10-min period before the CPA was removed in a reverse stepwise manner down the same osmotic ladder by transferring the slices down 1 M increments of descending CPA concentrations for 5 min each. Once the slices had been in the 1 M concentration well for 5 min, they were then immersed in X-VIVO for 5 min and then removed and placed in a final X-VIVO well for a minimum of 5 min in order to wash any remaining CPA before being prepared for viability staining. The maximal length of CPA exposure was 80 min for the 8 M solution (35 min to get to 8 M, 10 min at 8 M CPA testing concentration, and 35 min back down to 1 M), after which the slices were washed once in an X-VIVO solution well for 5 min, and then transferred to a second X-VIVO solution well where the slice remained until a total experimental exposure time of 90 min was obtained. For all concentrations less than 8 M, the slices were held in the second X-VIVO solution until the full 90 min was obtained. Positive controls involved placing two cartilage slices in a well containing X-VIVO solution for 90 min, while negative controls involved exposing the two cartilage slices to a solution containing 100% pure $Me_2SO$ for 90 min.

### *Chondrocyte viability staining and viability quantification*

A dual fluorescent membrane integrity assay was used to determine the viability of the chondrocytes within the slices. The slices were placed on a labeled microscope slide and stained with 40μL of a combination stain solution of Syto13 and ethidium bromide stain (8.6 μM/8.6 μM, Invitrogen, Carlsbad, CA/Sigma–Aldrich, St. Louis, MO). Syto13 is a cell membrane permeable nucleic acid specific stain that emits a green fluorescence and ethidium bromide (EB) is a cell membrane non-permeable stain specific for deoxy-ribonucleic acids that emits a red fluorescence. Therefore, cells with intact cell membranes emit a green fluorescence due to Syto 13 uptake and EB exclusion, while those with defective membranes emit a red fluorescence as EB is able to enter the cells. Once the combination stain solution was added to a cartilage slice, the microscope slide was allowed to incubate for 20 min at room temperature (~21 °C) in a dark environment. Chondrocyte membrane integrity was determined using fluorescent microscopy visualization using a fluorescence Nikon eclipse E600 with a dual filter (Omega optical cube TE 2000 with an Excitation of 480/25 and Emission of 530/640 wavelengths) and cell counts were obtained manually (Nikon Eclipse TE 2000-U fluorescent microscope, NIS Elements software, v. F 2.30, Nikon, Tokyo, Japan). Finally, chondrocyte recovery for each slice was normalized with respect to their own positive controls in order to account for losses due to patient, environmental, and harvesting factors.

Download English Version:

https://daneshyari.com/en/article/10928024

Download Persian Version:

https://daneshyari.com/article/10928024

Daneshyari.com