extracellular matrix and chondrocytes. However, CPA toxicity can induce chondrocyte death through mechanisms that include oxidative stress. Oxidative stress is produced through the significant imbalance between reactive oxygen species (ROS) and antioxidant species. This study investigated (1) the oxidative stress experienced by chondrocytes and (2) effects of antioxidant additives during chondrocyte in situ exposure to high CPA concentrations. We hypothesised that excessive ROS production and chondrocyte death during CPA exposure would be reduced by addition of antioxidant additives. Osteochondral dowels of 10 mm in diameter were harvested from femoral condyles of adult porcine stifle joints. Dowels were exposed to a multi-CPA solution [3 M dimethyl sulfoxide + 3 M ethylene glycol + 3 M propylene glycol] prepared in medium without additives, or supplemented with 0.1 mg/mL chondroitin sulfate (CS), or 2000 μM ascorbic acid (AA), at 4 °C for 180 minutes (N = 7). Cartilage slices from dowels were imaged using confocal microscopy after staining with either 5.0 μM dihydroethidium (DHE), a ROS fluorescent probe, or a dual fluorescent stain [6.25 μM Syto 13 + 9.0 μM propidium iodide] used to measure cell membrane integrity for viability quantification. A custom-made cell-counting program Viability 3.2 was used to quantify ROS concentrations and numbers of viable/non-viable cells. When compared to CPA without additives, CPA+CS and CPA+AA treatments resulted in significantly lower ROS ($p < 0.01$) and a lower decrease in percentage of viable cells ($p < 0.01$). The results suggest that CS and AA exhibit antioxidant properties that exert a protective effect on chondrocytes exposed to high concentrations of CPAs. Mitigating oxidative stress with antioxidants is one possible method of decreasing CPA toxicity, which can further improve future cryopreservation protocols.

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Conflict of Interest: Dr. N.M. Jomha and Dr. J.A.W. Elliott are co-inventors on US and Canadian patents for cryopreservation of articular cartilage, US Patent 8,758,988; Canada Patent 2,788,202.

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ADEQUACY OF POSTHYPERTONIC SHOCK MODEL TO REAL CRYOPRESERVATION CONDITIONS DURING DEGLYCEROLIZATION OF ERYTHROCYTES

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The research was to compare the efficiency of amphiphilic compounds belonging to different classes of surface-active substances under conditions of posthypertonic shock of human erythrocytes and at the stage of glycerol removal from cryopreserved cells. We used anionic sodium decyl sulfate, cationic trifluoperazine and nonionic decyl-β,D-glucopyranoside. Posthypertonic shock was initiated by transferring erythrocytes from dehydration (1.65 mol/L NaCl) into rehydration medium (0.15 mol/L NaCl) at 37 and 0°C. Erythrocyte suspension was frozen with glycerol (15%) by immersion into liquid nitrogen. To remove glycerol from warmed cells we used 0.6 mol/L (once) and 0.15 mol/L (twice) NaCl solution. Amphiphilic compounds were added into rehydration medium upon posthypertonic shock of erythrocytes and into second wash solution (0.15 mol/L NaCl) during deglycerolization of warmed cells. All studied amphiphilic compounds, regardless of their physicochemical properties and, as a consequence, the character of different transmembrane distribution under conditions of posthypertonic shock of erythrocytes at 0°C, were established to show quite a high antihemolytic activity (60–70%). At 37°C no protective effect of amphiphiles was revealed. Application of amphiphiles in deglycerolization of warmed erythrocytes enabled to reduce the level of cells hemolysis by 2–5 times, the most effective was nonionic decyl-β,D-glucopyranoside, which antihemolytic activity was ~ 90%. The research findings on the efficiency of studied amphiphilic compounds using in a model and real conditions of glycerol removal from warmed cells suggest a unified mechanism of their action. As well we hypothesize that it can be used for improvement of erythrocytes quality.

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CHARACTERISATION OF COLD-INDUCED MITOCHONDRIAL FISSION IN ENDOTHELIAL CELLS

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We have previously shown that hypothermia, 4°C as used for organ preservation, leads to mitochondrial fission in different cell types. Neither the temperature dependence and time course nor the mechanisms and consequences of cold-induced mitochondrial fission are currently known. Therefore, we here study cold-induced mitochondrial fission in endothelial cells, a cell type known to be very sensitive to preservation injury.

Cultured porcine aortic endothelial cells were incubated at 4°C (± additional temperatures) in Krebs-Henseleit (KH) buffer with glucose (5mM) and deferoxamine (1mM), the latter added to inhibit iron-dependent cold-induced injury.

Already after 3h at 4°C, endothelial mitochondria displayed a moderate degree of fission, pronounced fission was present after 24h (control: 67% of cells containing predominantly long mitochondria, 32% intermediate mitochondria; 24h 4°C: 1% long mitochondria, 46% short mitochondria). Cellular ATP content (control: 5.5±1.2nmol/106 cells) gradually declined to 1.8±0.7nmol/106 cells over 48h ($p<0.05$). Cold-induced fission occurred over a wide temperature range (≤20°C). At 4°C, the key fission enzyme Drp1 showed increased phosphorylation at both the activating (S616) and the inactivating (S637) site. The Drp1 inhibitor Mdivi-1 delayed but did not prevent cold-induced fission. Cleavage of the inner membrane fusion-mediating protein Opa1 to the fusion-incompetent s-Opa1 increased from 55% to 79%.

After cold incubation in KH buffer, cold-induced fission was reversible after rewarming (37°C) in cell culture medium, even after 48h of hypothermia; in parallel, ATP increased again. Rewarming temperatures required for >50% re-fusion and ATP regeneration were ≥25°C. However, after cold incubation in a low chloride modification of KH buffer – to simulate preservation conditions – mitochondria remained fragmented, ATP levels did not recover and cell death followed.

Cold-induced mitochondrial fission appears to be unavoidable at low temperature, important for endothelial function/injury after exposure to preservation conditions and mechanistically complex. Understanding its mechanisms is desirable for the optimization of organ reconditioning protocols.

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VARIATIONS IMPACTING CELLULAR OUTCOME DURING RETENTION AND THAWING OF HUMAN APHERESIS SAMPLES

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