Impact of Normothermic Perfusion and Protein Supplementation on Human Endothelial Cell Function During Organ Preservation

Thomas Puehler, MD, Otto Gleich, PhD, Simon Schopka, MD, Leopold Rupprecht, MD, Stephan Hirt, MD, Christof Schmid, MD, and Karla Lehle, PhD

Department of Cardiothoracic Surgery, and Ear, Nose, and Throat Department, University Medical Center Regensburg, Regensburg, Germany

Background. **Hypothermia-induced changes in endothelial cell (EC) morphology and function after organ storage may influence the initial outcome and development of transplant-associated coronary artery disease.**

Methods. **Human saphenous vein ECs were incubated with saline (NaCl), University of Wisconsin (UW), and histidine-tryptophan-ketoglutarate (HTK) solution, with and without protein additives, at 4°C and 37°C. After 6 hours, ECs were recultivated for 24 and 48 hours with culture medium (reperfusion). Mitochondrial activity, adenosine triphosphate concentration, cell count, and inflammatory responses were analyzed.**

Results. **Cold preservation did not affect the mitochondrial activity of ECs and allowed a complete regeneration of the metabolic turnover after reperfusion. However, under normothermic conditions the metabolism of the cells was influenced by time and type of preservation solution. While both the mitochondrial activity and cell count did not change after treatment with NaCl and culture medium, the metabolic turnover of cells treated with HTK and UW solution significantly increased (twofold) and decreased (twofold, $p < 0.05$), respectively, after reperfusion. The endothelial reactivity remained unchanged after treatment with NaCl and HTK. The addition of serum proteins significantly improved mitochondrial activity of cells treated with warm NaCl and HTK ($p < 0.05$). The UW-treated cells burned out through a significant up-regulation of the ATP concentration resulting in a complete metabolic regression after reperfusion and induction of apoptosis.**

Conclusions. **Normothermic preservation in UW prevented regeneration of ECs, while treatment with HKT solution did not irreversibly affect mitochondrial activity of ECs and allowed complete regeneration of metabolism and function. Serum proteins improved the preservation effect of HTK and NaCl.**

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Cold preservation is the standard procedure for organ transplantation to minimize hypoxic injury during ischemia time [1]. However, hypothermia causes direct deleterious cellular effects and injury through a number of pathways that occur upon reperfusion and increase with as the duration of cold preservation increases [2, 3]. The cellular compartment most sensitive to preservation injury is the endothelial cell (EC) [4, 5], which is also the first target during reperfusion. As a consequence, cellular swelling, platelet accumulation, impairment of procoagulant and anticoagulant properties, and leukocyte adherence [3] might cause worsened initial outcome after heart transplantation (HTx) [6]. In this context, improvements of preservation solutions are one opportunity to minimize changes in cellular homeostasis, membrane barrier function, and cell volume during cold storage [1, 2, 7–9]. Cold University of Wisconsin (UW) [10] and Bretschneider's histidine-tryptophan-ketoglutarate [11] solutions are commonly used for cardiac preservation. While in most studies UW was rated superior for EC preservation during hypoxic storage [6, 12, 13], the subsequent rewarming-reoxygenation exhibited an increase in the expression of inflammatory and stress proteins [13], leading to cold-induced cell volume changes and imbalances in cellular ion homeostasis [14]. Furthermore, cold storage in HTK also disturbed the EC integrity [15].

A "new paradigm for organ preservation" [16] favored warm preservation to maintain physiologic temperature and organ function during preservation for a successful transplantation. Brockmann and colleagues used a pig liver transplant model to show that warm perfusion under physiologic pressure and flow enabled prolonged preservation and successful transplantation. This model is based on the preservation of the liver, but it is likely to be applicable to other organs [16]. Other novel preservation techniques include the optimization of the solution composition with additives [8, 9, 17], and the introduction of normothermic blood perfusion preservation, permitting the maintenance of normal cellular metabolism [18]. So far, the benefit of normothermic preservation was only documented in experimental studies [17, 18] and has not achieved widespread clinical applicability [18].

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Address correspondence to Dr Puehler, Department of Cardiothoracic Surgery, University Medical Center, Regensburg, D-93042, Germany; e-mail: thomas.puehler@klinik.uni-regensburg.de.

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Abbreviations and Acronyms

ATP = adenosine triphosphate
BSA = bovine serum albumine
CMS = culture medium with 10% human serum
DAPI = 4,5-diamidino-2-phenylindole
DMSO = dimethylsulfoxid
EC = endothelial cells
EDTA = ethylendiamin-tetraacetat
HS = human serum
HSVEC = human saphenous vein endothelial cells
HTK = histidine-tryptophan-ketoglutarate
HTX = heart transplantation
MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4 sulfophenyl)-2H-tetrazolium, inner salt
NaCl = sodium chloride
PBMC = peripheral blood mononuclear cells
PBS = phosphare buffered sialine
SD = standard deviation
TNF = tumor necrosis factor
UW = University of Wisconsin solution

In our experimental study, we optimized standard cardiac preservation solutions by the addition of plasma proteins and used normothermic preservation conditions to avoid cold shock-induced injury of EC during standard organ preservation. The primary outcome of our study was the preservation of EC metabolism, morphology, and function.

Material and Methods

Cell Culture and Experimental Protocol

We obtained saline (NaCl) from Baxter (Unterschleißheim, Germany), UW from DuPont (Bad Homburg, Germany), and HTK from Dr. Köhler Chemie (Alsbach, Germany). After informed consent of the patients and agreement by the local ethics committee, human ECs were derived from saphenous veins (HSVEC) using standard isolation procedures [19, 20]. Cells were cultured in CMS (culture medium with 10% human serum; Cat.No. 2010111, Provitro, Berlin, Germany) on 0.1% gelatin-coated (Sigma, Munich, Germany) tissue culture flasks (Falcon, Heidelberg, Germany). Cells in passage 1 were deep-frozen in 10% dimethylsulfoxide (Gibco, Karlsruhe, Germany) and recultured before the experiment started. The HSVEC were characterized by their "cobblestone morphology" and factor VIII-related antigen expression [19]. The HSVEC (4.000 cells/cm^2) were grown for 5 days in 96-well plates (Falcon). For preservation conditions, HSVEC were carefully washed and incubated in UW, HTK, NaCl (0.9 %), and CMS (= control) for 2 to 6 hours at 4°C and 37°C, respectively. After a 6 hour preservation, prewarmed CMS was added for another 24 (= 6 + 24) and 48 (= 6 + 48) hours under standard culture conditions (= reperfusion). The latter was defined as the regeneration of the metabolic turnover. At each time point, the level of mitochondrial activity [21], the absolute cell count, and the adenosine triphosphate (ATP) content [22] were measured (see Appendix to Materials and Methods).

Furthermore, the impact of different additives (bovine serum albumin [BSA], Sigma; HS, human serum) to the preservation solutions was evaluated analyzing the mitochondrial activity. In addition, the influence of selected preservation solutions on the endothelial function (expression of cellular adhesion molecules, adhesion of peripheral mononuclear blood cells) and the apoptosis induction (apoptotic bodies) was checked. Details on the test systems and the experimental protocol were presented in the Appendix.

Statistics

Data are expressed as mean ± standard deviation (SD). The statistical analysis was performed using the software packages SPSS15 (SPSS Inc, Chicago, IL) and SigmaStat

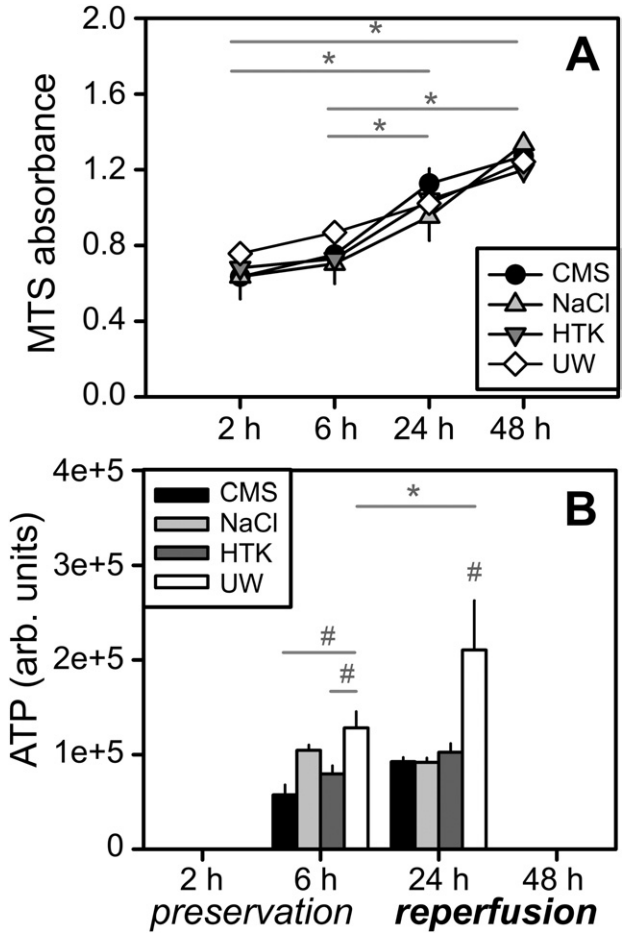

Fig 1. Impact of cold preservation on the mitochondrial activity (A) and the ATP content (B) in HSVEC. The HSVEC were pretreated with CMS (= control), NaCl, UW, and HTK for 2 and 6 hours (preservation). After 6 hours solutions were replaced with CMS and cells were cultivated under standard culture conditions (24 and 48 hours reperfusion). The MTS absorbance and ATP content was determined as described in the Appendix. ($p < 0.05$ comparing different incubation times; # comparing different solutions.)*