Coronary oxygen persufflation combined with HTK cardioplegia prolongs the preservation time in heart transplantation

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Abstract

\textbf{Background:} One of the most restricting factors remaining in heart transplantation is the limited myocardial ischemia time. A new approach towards the prolongation of this time is the combination of primary cardioplegic arrest followed by continuous coronary oxygen persufflation (COP) with gaseous oxygen. \textbf{Methods:} This technique was applied in pig hearts, which we transplanted orthotopically after cardioplegic arrest by original \textit{n} = 5 and modified (addition of hyaluronidase: \textit{n} = 11) Bretschneider HTK solution and 14 h of hypothermic preservation. Depending on the different preservation techniques, we created four groups: (1), original HTK (HTK), \textit{n} = 5; (2), modified HTK (mHTK), \textit{n} = 5; (3), modified HTK solution plus COP (mHTK + COP), \textit{n} = 6; and (4), as a control five hearts were transplanted after cardioplegic arrest by the original HTK solution and a cold ischemia time of 3 h comparable to clinical routine procedure. \textbf{Results:} After 14 h of preservation and orthotopic transplantation, cardiac functional recovery in mHTK + COP hearts was similar to control hearts, and improved compared to hearts of both other groups. Hemodynamics were significantly better in hearts preserved by mHTK + COP and in the control group compared to the HTK-hearts (\textit{P} < 0.05), not significant compared to mHTK hearts (dp/dt\textsubscript{max} in \% of preoperative \pm standard error of mean (SEM): mHTK + COP, 85 \pm 9; control, 85 \pm 10.5; mHTK, 59 \pm 14; HTK, 50 \pm 4). The cardiac output (CO) in \% of preoperative was: mHTK + COP, 68 \pm 5.4; control, 64 \pm 4; mHTK, 44 \pm 2.7; HTK, 25 \pm 11. The ATP of left ventricular myocardium in mHTK + COP hearts at 14.7 \pm 1 \mu mol/g dry weight (DW) and in the control at 14.59 \pm 1.8 was higher compared to that in mHTK at 12.2 \pm 2.8 (\textit{P} is non-significant (n.s.) versus mHTK + COP and control) and in HTK-hearts at 7.0 \pm 0.5 (\textit{P} < 0.05 versus mHTK + COP and control). CK–MB in percent of CK showed no increase in either group. \textbf{Conclusions:} These data show that COP combined with a mHTK solution represents a potential alternative to complement currently used cold storage techniques for prolonged preservation periods. © 2000 Elsevier Science B.V. All rights reserved.

\textbf{Keywords:} Coronary oxygen persufflation; Heart transplantation; Crystalloid cardioplegia; Prolonged heart preservation; Hypothermia

1. Introduction

In heart transplantation, the preservation time of explanted organs remains a restricting problem. Many attempts have been undertaken to prolong ischemia time; experimentally successful in vitro reperusions were performed after ischemia times of up to 24 h [1]. Continuous perfusion of explanted hearts was performed in the past [2]. These findings showed little consequence on human heart transplantation. Currently, ischemia time in clinical routine procedures is limited to 3–5 h using flush perfusion and cold storage solution techniques [3]. In an attempt to prolong the myocardial preservation time, we experimentally investigated the continuous coronary oxygen persufflation (COP) [4], which implicates continuous antegrade persufflation of the coronary arteries with gaseous oxygen after cardioplegic arrest with crystalloid Bretschneider’s HTK solution. This solution was modified by the addition of hyaluronidase (40 mg/l), which resulted in superior cardioprotective qualities compared to unchanged HTK solution with cold storage of up to 24 h in rat and rabbit hearts [5]. The technique of antegrade or retrograde gaseous coron-
ary oxygen persufflation has shown in the past [6–8] to maintain myocardial function without blood supply for a time of up to 12 h. These experiments were performed at normothermia and have thus far shown no influence, either on cardiac surgery routine procedures or on cardiac transplantation.

Oxygen persufflation has been used even for experimental kidney and liver preservation. A significant improvement of functional and metabolic outcome could be demonstrated [9–11].

The aim of our study on mature pigs, was to investigate whether the continuous oxygen persufflation (COP) on the basis of modified HTK (mHTK) solution, which has been shown to be superior to the University of Wisconsin (UW) and Euro-Flush solution with glutathione (EFG) storage [4], also improves the recovery compared to hearts preserved by HTK or mHTK storage during 14 h without COP, respectively compared to hearts with only 3 h cold storage in HTK solution.

2. Materials and methods

All animals were housed, fed and handled in compliance with German legislation on protection of animals (approval by the ‘Regierungspräsident Köln’) and the ‘Guide and Use of Laboratory Animals’ published by the NIH (publication number 86–23, revised 1996).

2.1. Operative technique

After premedication and iv anaesthesia, 21 pigs (Duroc–German Landrace hybrids, halothan-resistant, 28.9 ± 1.1 kg body weight (BW)) were placed in the supine position and endotracheally intubated. Ventilation was performed with 50% oxygen and 50% nitrous oxide (N2O) using a volume-cycled respirator (Engström RS 300, LKB Medical, Bromma, Sweden). The common carotid artery and the internal jugular vein were cannulated for measurement of arterial and central venous blood pressure. Access to the heart was obtained by median sternotomy.

2.2. Hemodynamic parameters and LV function

The following parameters were measured as baseline: arterial (AP) and central venous (CVP) blood pressure; heart rate (HR); left ventricular pressure (LVP); and dp/dt by a micro-tip catheter pressure transducer. Cardiac output (CO) was measured by thermodilution.

2.3. Cardioplegia and storage

After occlusion of both the caval and left azygos (which leads into the coronary sinus) veins the ascending aorta was cross-clamped, and cardioplegic arrest was achieved by antegrade infusion of cold (0–1°C) cardioplegia solution at a pressure of 75 mmHg for the first min and 40 mmHg for the following 9 min. Ten hearts received original Bretschneider–HTK solution (Custodiol®, Dr F. Köhler Chemie GmbH, Alsbach–Hähnlein, Germany), and 11 hearts received mHTK solution (addition of 40 mg/l hyaluronidase, Boehringer, Mannheim, Germany) [12]. Hearts were then explanted and stored at 0–1°C in the respective solution. Additionally, six pig hearts of the mHTK-group were submitted to continuous antegrade coronary oxygen persufflation (mHTK + COP) at a pressure of 45 mmHg and a flow rate of 80 ± 10 ml/min (Fig. 1).

2.4. Orthotopic transplantation

Recipient pigs with a BW of 27.1 ± 1.0 kg were placed and prepared as described above.

Sonomicrometry (Sonometrics Corp, London, Ontario, Canada) and micromanometry (Millar Instruments Inc., Houston, TX) were applied to determine the preload recruitable stroke work (PRSW) [13].

Blood samples for CK (CK–NAC monotest, Boehringer, Mannheim, Germany) and CK–MB (agarose gel electrophoresis, Helena Laboratories, Beaumont, TX) baseline measurements were taken.

Heparin was given before cannulation of the aorta and both venae cavae for standard cardiopulmonary bypass (CPB). The roller pump equipped heart-lung machine...
(HLM-CAPS, Stöckert Instruments, Munich, Germany) with a membrane oxygenator (Maxima, Medtronic GmbH, Düsseldorf, Germany) was primed with whole blood. Prior to excision of the hearts, blood samples for enzyme measurements (CK, CK–MB) and LV biopsies (as baseline measurements) were taken from the recipient hearts for measurement of adenine nucleotides, creatine phosphate, free creatine, glycogen and lactate, using enzymatic tests and high performance liquid chromatography (HPLC). All values of intracellular substances (except lactate, calculated/g Tissue wet weights (WW) were calculated/g tissue dry weight (DW) after lyophilization, which has been corrected for residual water content in heat-drying experiments.

Recipient hearts were then excised and donor hearts orthotopically implanted as described by Lower and Shumway [14]. Pigs were temporarily cooled down to 20°C. Additional topical cooling with cold saline and cooling jacket (Cobe Cardiovascular Laboratories Inc, Arvada, CO) was performed. The storage time including implantation procedure was 14.5 ± 0.1 h (mean ± standard error of mean (SEM)); HTK, 14.6 ± 0.2; mHTK, 14.5 ± 0.2; and mHTK + COP, 14.4 ± 0.1 non-significant (n.s.); and 3.3 ± 0.1 h in the control group. Prior to opening the aortic cross clamp (except in the control group), a 10-min perfusion via the ascending aorta with warm (37°C), modified Krebs–Henseleit solution at a perfusion pressure of 50 mmHg was performed (Table 1). Ca²⁺ content in this solution was subsequently augmented from 0.05 to 1 mmol/l after 10 min. The implanted hearts were then reperfused on CPB with whole blood starting at a mean pressure of between 40 and 60 mmHg for 118 ± 10 min. The implanted hearts were then reperfused on CPB was subsequently augmented from 0.05 to 1 mmol/l after 10 min. The implanted hearts were then reperfused on CPB with whole blood starting at a mean pressure of between 40 and 60 mmHg for 118 ± 10 min. (HTK, 122 ± 26; mHTK, 125 ± 28; mHTK + COP, 106 ± 2; and control, 121 ± 24 min, n.s.). During the first h of reperfusion, adenosine (13.5 μmol/min) was administered into the aortic root, and the end to end pulmonary artery anastomosis was performed. For immunosuppression all animals received 500 mg methylprednisolone (Urbason soluble, Hoechst Pharma A.G., Frankfurt, Germany) i.v. prior to the opening of the aortic cross clamp. Blood samples for CK and CK–MB measurements were taken every 30 min. Pigs were then weaned off CPB without inotropic support. Hemodynamic measurements were performed after a period of 40 ± 6 min of reperfusion without CPB on average. Prior to the termination of the experiments (according to the terms of the governmental permit) LV biopsies were taken for measurements of adenine nucleotides, creatine phosphate, free creatine, glycogen and lactate, as described above. The energy charge potential (ECP) was calculated as follows: ECP = (ATP + 1/2ADP)/(ATP + ADP + AMP). DW from left ventricular biopsies (1 cm³) was measured after lyophilisation with correction for residual water. Myocardial water content (MWC) was derived as ((WW–DW)/WW) and expressed as percent of WW.

2.5. Statistical analysis

Data are presented as mean values and SEM. Comparison of mean values was performed by the use of analysis of variance (ANOVA) followed by the Bonferroni t-test for comparison of differences between several groups. Statistical significance was assumed where P is less than 0.05.

3. Results

After weaning from CPB, hearts of the mHTK + COP group and control hearts showed significantly better values for left ventricular pressure (LVP), dp/dt max (Fig. 2), dp/dt min and CO (Fig. 3) compared to HTK hearts, but n.s. compared to mHTK hearts.

In contrast to the persulfated (69 ± 6, P < 0.05 versus HTK and mHTK) and control hearts (46 ± 8 min, n.s. versus HTK and mHTK), HTK and mHTK hearts were not able to maintain a stable circulation for longer than 15 ± 11 and 25 ± 10 min, respectively, without CPB support. Three HTK-hearts and two mHTK-hearts showed visible alterations in myocardial structure in terms of hypoperfused areas, subepicardial hematomas and myocardial contractures. These hearts did not improve under the administration of catecholamines.

Pregrafting PRSW was 58.9 ± 8.7 mmHg, and post transplantation including reperfusion was 51.9 ± 9.1 mmHg in the mHTK + COP group. In the other groups, PRSW values could not be measured due to arrhythmias and ventricular dyskineties.

Postoperative high energy phosphates showed a decrease in all hearts compared to pregrafting values of normal recipient hearts; however, ATP content in mHTK + COP and control hearts was higher compared to hearts of the HTK-group (P < 0.05) and mHTK hearts (n.s., Fig. 4). ECP values amounted to the following: normal recipient hearts, 0.90 ± 0.00; mHTK + COP, 0.87 ± 0.01; control, 0.87 ± 0.02; mHTK, 0.88 ± 0.01; HTK, 0.85 ± 0.02. Glycogen stores showed no significant differences between groups:

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition of the modified Krebs–Henseleit solution with low initial Ca²⁺ content, high glucose levels and the addition of adenosine, uric acid and insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substancesa</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Na⁺</td>
<td>143.1</td>
</tr>
<tr>
<td>K⁺</td>
<td>5.9</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.05</td>
</tr>
<tr>
<td>Mg²⁺</td>
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</tr>
<tr>
<td>Cl⁻</td>
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<tr>
<td>HCO₃⁻</td>
<td>10.1</td>
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<tr>
<td>H₂PO₄⁻</td>
<td>1.2</td>
</tr>
<tr>
<td>SO₄²⁻</td>
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</tr>
<tr>
<td>Adenosine</td>
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</tr>
<tr>
<td>Glucose</td>
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</tr>
<tr>
<td>Uric acid</td>
<td>1.0</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.0b</td>
</tr>
</tbody>
</table>

a Continuous oxygenation with 95% O₂ and 5% CO₂.
b The measurement unit for this insulin value is (IU/l).
After 3 h of reperfusion dp/dt\textsubscript{max} of the persulfated and control hearts was significantly higher compared to the HTK group. Heart rate was 98 ± 4 beats/min preoperatively without differences between groups; postoperatively, 129 ± 7 (P < 0.05 vs. preoperative) with a significantly higher rate of the control hearts (167 ± 5 versus the other groups). Left ventricular enddiastolic pressure was 7 ± 0.5 mmHg preoperatively and 11 ± 0.7 postoperatively, without differences between groups. HTK, Bretschneider’s HTK cardioplegia; mHTK, mHTK (addition of hyaluronidase); mHTK + COP, mHTK combined with continuous coronary oxygen persufflation; control, Bretschneider’s HTK cardioplegia, and 3 h ischemia. #P < 0.05 vs. before grafting; *P < 0.05 vs. mHTK + COP and control. Values are mean ± SEM.

mHTK + COP, 116 ± 9; control, 88.2 ± 27; mHTK, 157 ± 29; and HTK, 91 ± 13 μmol/g DW. The pregrafting value was 102.6 ± 1.8 μmol/g DW. Lactate levels of mHTK + COP (9.2 ± 1.5 μmol/g WW) and control-hearts (8.2 ± 1.5) were significantly elevated compared to pregrafting values; 4.2 ± 0.9 in contrast to HTK (6.3 ± 0.8) and mHTK hearts (5.0 ± 0.5). Except for the mHTK + COP hearts, myocardial water content (MWC) increased significantly after transplantation and reperfusion compared to pregrafting pig hearts (80.7 ± 0.2%). Values in percentages amounted to mHTK + COP, 80.9 ± 0.3; control, 81.9 ± 0.7; mHTK, 81.6 ± 0.2; and HTK, 85 ± 0.4 (P < 0.05 versus mHTK + COP, control, mHTK). CK−MB/CK values did not exceed 10% (Fig. 5).

4. Discussion

With this data the beneficial effect of COP on myocardial preservation could be demonstrated. Functional and metabolic parameters after 14 h of storage, transplantation and reperfusion were similar to values of the control hearts with an ischemia time comparable to clinical routine procedures.

4.1. Reduction of anoxia

Even though hypothermia reduces myocardial metabolism [15], oxygen deficiency with consecutive depletion of energy-enriched phosphates still occurs [16–19], which we try to prevent by the application of COP.

However, due to the fact that measurements of the metabolic situation in our pig heart model could only be performed after reperfusion, a definitive statement that coronary oxygen persufflation would better maintain an
aerobic metabolism is not jet admissible. On the other hand, we could show recently in unpublished experiments on rabbit hearts that aerobic metabolism and lactate were normal directly after 14 h of perfusion. Therefore, elevated lactate levels in the perfused and control hearts compared to both other groups and pregrafting values must be an effect of reperfusion. The supposition of a marked ischemia in both of these groups is inconsistent with the good functional and metabolic measurements of the perfused and control hearts. Myocardial lactate levels could be influenced by systemically augmented lactate concentrations during reperfusion and possibly reflect a better myocardial perfusion of the perfused and control hearts compared to HTK and mHTK hearts, whose lactate levels did not increase after reperfusion despite worse myocardial function.

The coronary effluent of lactate could not be measured due to an extremely vulnerable tissue of the coronary sinus with the risk of perforation by a catheter.

CK–MB/CK ratios below 10% in all groups indicate that no major damage occurred, even though augmented values in the HTK and mHTK hearts during late reperfusion are supposed to indicate minor injury which would be convenient to visible myocardial changes observed postoperatively.

4.2. Avoidance of edema

Avoidance of edema might also present an important factor for the excellent myocardial recovery in COP hearts as edema per se is known to impair myocardial function [20]. The main effect of edema reduction of the mHTK + COP and the mHTK hearts compared to HTK hearts is probably due to the addition of hyaluronidase to the cardioplegia, since it is known to reduce intercellular hyaluronic acid concentration, and herewith, fluid accumulation [12,21,22].

4.3. Controversies

Air embolism after oxygen perfusion was prevented by perfusion of the coronary arteries with a low viscosity electrolyte solution prior to blood reperfusion.

Another major problem is related to the potentially deleterious effect of oxygen on the endothelial wall. Despite the good functional and metabolic results of the perfused hearts, which could be an indicator of a functioning endothelium, we will focus our future investigations on this issue. However, preliminary results of our investigations on hearts after COP and 7 days of in vivo reperfusion show well functioning coronary endothelium (measurement of the endothelium-dependent relaxation) and morphological preservation of the endothelial layer and myocytes (electron microscopy).

Despite these encouraging initial results, further investigations about long-term myocardial and endothelial function, particularly the morphology of hearts preserved with the COP technique, are now conducted in our laboratory prior to clinical application.

Acknowledgements

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