Orthotopic transplantation of pig hearts harvested after 30 min of normothermic ischemia: controlled reperfusion with blood cardioplegia containing the Na\(^+\)-H\(^+\)-exchange inhibitor HOE 642

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Abstract

Objectives: The aim of our study was to develop a surgical technique for a successful transplantation of hearts harvested after 30 min of normothermic ischemia without donor pretreatment. Successful transplantation of ischemic compromised hearts could help to expand the severely limited donor pool. We used the pig model because this species is very susceptible to myocardial ischemia. Na\(^+\)-H\(^+\)-exchange (NHE) inhibitors have shown excellent protective properties in several in vitro and in vivo models of myocardial ischemia and reperfusion.

Methods: In group I (n = 12) hearts were harvested after 30 min of normothermic ischemia following cardiac arrest induced by exsanguination. Hearts were perfused with warm blood cardioplegia and transplanted orthotopically. In group II (n = 9) controlled reperfusion with cold leucocyte-depleted blood cardioplegia was performed after 30 min of normothermic ischemia. In group III (n = 8) the same procedure was performed as in group II but blood cardioplegia contained 1 mmol/l HOE 642.

Results: In group I massive myocardial oedema was observed and none of the animals could be weaned from cardiopulmonary bypass (CPB). In contrast, all animals in groups II and III could be weaned from CPB with low dose inotropic support. In groups II and III the contractility of the hearts, expressed as maximal left and right ventricular stroke work index was significantly impaired after transplantation as compared with the preoperative value. Supplementation of blood cardioplegia with HOE 642 resulted in a significantly better recovery of the LVSWImax (Group II vs. III).

Conclusions: Successful transplantation of pig hearts is possible after 30 min of normothermic ischemia without donor pretreatment if a controlled reperfusion with cold leucocyte-depleted blood cardioplegia is performed. HOE 642 given during reperfusion only improves posttransplant left ventricular function.

Keywords: Heart transplantation; Myocardial preservation; Non-heart-beating donors; Controlled reperfusion; Na\(^+\)-H\(^+\)-exchange-inhibitors

1. Introduction

Currently, the art and science of cardiac transplantation have progressed to the point where donor availability is the major problem. To meet the donor shortage, enormous research efforts are undertaken. Encouraged by the clinical experience with organ donation from non-heart-beating donors (NHBD) in kidneys [1], discussion on the use of ischemically damaged hearts for heart transplantation is in progress. In NHBD organ preservation is initiated some time after circulatory arrest, so that the organs are exposed to normothermic ischemia. The heart, however, is more susceptible to normothermic ischemia than the kidney or the liver. Irreversible damage to myocardial tissue occurs after 20 min of normothermic ischemia, if unmodified blood reperfusion is used [2].
Therefore, refinements of myocardial protection strategy are necessary in these severely damaged hearts. Reperfusion injury can be reduced when substrate-enriched warm blood cardioplegia is given at low perfusion pressure [3,4].

The purpose of our study was to evaluate if a successful transplantation of hearts after 30 min of normothermic ischemia is possible. We used pigs due to the similarity of porcine myocardium to the human [5]. However, the porcine heart is more susceptible to ischemia than the heart of other laboratory animals. In an isolated heart model, pig hearts harvested 10 min or more after animals exsanguination failed to be successfully reanimated [6].

We used a Na+–H+–exchange (NHE) inhibitor since previous investigations revealed the importance of the NHE in myocardial ischemia and reperfusion [7]. Inhibition of NHE can avoid 

\[ \text{Ca}^{2+} \]

overload of the myocardium during ischemia and reperfusion and prevent irreversible cellular damage. NHE inhibitors have shown excellent cardioprotective and anti-arrhythmic effects in different in vitro and in vivo models of myocardial ischemia and reperfusion [7].

2. Materials and methods

2.1. Animals and anesthesia

All procedures were performed in conformity with the ‘Guide for the Care and Use of Laboratory Animals’ and the German ‘Law on the Protection of Animals’. Pigs of the ‘German Landrace’ weighing 18–31 kg (mean 25 ± 4 kg) were premedicated with an intramuscular injection of 0.2 mg/kg flunitrazepam and 7 mg/kg ketamine hydrochloride. An ear vein was cannulated and anesthesia was induced with 0.1 mg/kg flunitrazepam and 10 mg/kg ketamine hydrochloride by intravenous injection. An endotracheal tube was inserted and mechanical ventilation begun. Catheters were introduced into the carotid artery and internal jugular vein to measure arterial pressure, central venous pressure, pulmonary artery pressure and cardiac output.

2.2. Surgical procedure

After median sternotomy 500 IU/kg heparin were given to all pigs. A 7F polyurethane catheter was inserted into the left atrium through the left atrial appendage to monitor left atrial pressure. A 12 gauge cannula was placed into the ascending aorta of the donor hearts for application of cardioplegia. This cannula was also used for exsanguination of the animals. After procurement the donor hearts were stored in ice-cold cardioplegic solution for 1 h.

2.3. Cardiopulmonary bypass (CPB)

The heart lung machine (Stöckert, München, Germany) was filled with a priming of 1000 ml of hydroxyethyl starch. We used a pediatric oxygenator (D 705, Dideco, Mirandola, Italy), systemic heater/cooler (Jostra, Hirlingen, Germany), and an arterial filter (D 733, Dideco, Mirandola, Italy). Cannulation of the ascending aorta was performed using a 16F aortic cannula. Cannulation of the superior and inferior vena cava was achieved through the right atrium with 20F and 28F cannulas. A 9F vent catheter was inserted into the left atrium to avoid distention of the left ventricle. The blood flow was 2.2–2.5 l/min.

2.4. Orthotopic heart transplantation

Transplantation was performed in a biatrial technique. The left azygous vein, a special feature of the porcine anatomy, was dissected in the donor, left azygous vein and coronary sinus of the recipient remained intact. At the end of the experiment the animals were sacrificed by an intravenous injection of potassium chloride.

2.5. Experimental groups

In group I (transplantation after 30 min of normothermic ischemia, controlled reperfusion with warm blood cardioplegia, \( n = 12 \)) hemorrhagic shock was induced by exsanguination. After zero blood pressure the animals were left undisturbed for 30 min. Myocardial temperature averaged 33°C. After aortic cross-clamping warm (37°C) blood cardioplegia (BCP) for controlled reperfusion (Dr. Franz Köhler Chemie GmbH, Alsbach-Hähnlein, Germany) containing 500 mg/l methylprednisolone was given for 15 min at a perfusion pressure of 35–40 mmHg. Then the heart was excised, placed in ice-cold (4°C) BCP for 1 h and transplanted orthotopically. After implantation a second controlled reperfusion with BCP (37°C, 40 mmHg) was performed for 5 min. After the release of the aortic cross-clamp the heart was kept in a beating empty state for at least 1 h before it was weaned from CPB. In case of unstable hemodynamic conditions bypass time was prolonged. For inotropic support adrenaline (0.1 μg/kg per min) was given to all animals.

In group II (transplantation after 30 min of normothermic ischemia, controlled reperfusion with cold leukocyte-depleted BCP, \( n = 9 \)) cardiac arrest was induced as in group I. After 30 min of normothermic ischemia leukocyte-depleted BCP (4°C, blood cardioplegic solution for controlled reperfusion, Dr. Franz Köhler Chemie GmbH) was given. Transplantation was performed following the same procedure as in group I. After implantation a second controlled reperfusion with leukocyte-depleted BCP (20°C, 40 mmHg) was performed for 5 min. This perfusion was continued with leukocyte-depleted, non-cardioplegic blood for 30 min at a perfusion pressure of 40 mmHg continuing aortic cross-clamping. The temperature of the blood was 20°C at the beginning of the reperfusion and was increased stepwise to 37°C over the 30 min period.

In group III (transplantation after 30 min of normothermic ischemia, controlled reperfusion with cold, leukocyte-
depleted BCP containing HOE 642, \( n = 8 \) the same procedure was performed as in Group II, but BCP for the first and second controlled reperfusion was supplemented with 1 mmol/l HOE 642 (Hoechst AG, Frankfurt/M., Germany). The overall dose of HOE 642 in the recipient was limited to 2 mg/kg.

2.6. Hemodynamic measurements

Hemodynamic examinations were performed before procurement of the donor hearts and 1 h after weaning from CPB. Cardiac output was measured by the thermodilution technique. Cardiac preload was increased by stepwise volume loading, controlling left atrial pressure and Starling curves were registered. Left and right ventricular stroke work were calculated and normalized for heart weight as stroke work index:

\[
LVSWI (mJ/g) = \frac{MAP - LAP (mmHg) \times CO(l/min) \times 0.133}{HR (beats/min) \times HW (g)}
\]

\[
RVSWI (mJ/g) = \frac{PAP - CVP (mmHg) \times CO(l/min) \times 0.133}{HR (beats/min) \times HW (g)}
\]

where, \( LVSWI \) is left ventricular stroke work index; \( RVSWI \) is right ventricular stroke work index; \( MAP \) is mean aortic pressure; \( PAP \) is mean pulmonary artery pressure; \( LAP \) is mean left atrial pressure; \( CVP \) is central venous pressure; \( HR \) is heart rate and \( HW \) is heart weight.

To compare the contractility in the experimental groups we used the maximal achieved left and right ventricular stroke work index (LVSWImax and RVSWImax, respectively).

2.7. Heart weight

Heart weight was determined after the excision of the donor heart and at the end of the experiment.

2.8. Enzymes

Myocardial fraction of creatin kinase (CK-MB) and lactate dehydrogenase (LD 1) were measured. With regard to the peculiarity of the porcine isoenzymes, we used an agarose electrophoresis test (REP-CK/LD-isoenzyme combo method, Helena Laboratories, Beaumont, TX), which determines the CK-MB and the myocardial fraction of lactate dehydrogenase (LD 1). This test analyzes the percentage of CK-MB and LD 1, in relation to the total CK and total LD, respectively. To calculate the absolute values for CK-MB and LD 1, total CK and LD measurement was necessary. Total CK was measured photometrically with an enzymatic test (CK (NAC) AU 5000 Analyser System, Merck, Darmstadt, Germany), total LD values were determined by means of an enzymatic test (LDH Merck MEGA, Merck, Darmstadt, Germany).

2.9. White blood cell count

To evaluate the efficiency of the leucocyte filter, white blood cells were counted at the beginning of reperfusion, after 5 and after 10 min in 11 experiments. Blood samples were taken from the cardioplegia line before and after BCP had passed the leucocyte filter.

2.10. Postmortem macroscopic and histological examination

Two hours after the end of CPB macroscopic inspection of the donor hearts was performed. Samples of the anterolateral left ventricular wall were fixed in 10% formalin, embedded in paraffin, cut and stained with hematoxylin and eosin. Microscopic examination was performed by one blinded observer.

2.11. Statistical analysis

Statistical analysis was performed with a statistical computer program (Graph Pad Software, San Diego, CA). For comparison of pre- and posttransplant values and analysis of the CK and LDH isoenzymes, we used the paired \( t \)-test with a two-tail \( P \) value. Hemodynamic data of groups II and III were compared by an unpaired \( t \)-test using Welch’s correction. Data of all the three groups were compared by analysis of variance (ANOVA), for significance testing between the different groups we used the Bonferroni correction. Group statistics were expressed as mean ± standard deviation. The variable \( n \) in the tables and figures represents the number of experiments data could be obtained from.

3. Results

Results are summarized in Table 1 and Figs. 1–5. Eight animals had to be excluded from the data analysis due to technical, surgical or procedural problems. In six cases the hemoglobin level after transplantation had decreased to less than 6 g/dl caused by excessive bleeding. Two animals died because of technical problems with the extracorporal circuit, resulting in air embolism and a blood temperature of more than 42°C.

The time to perform the implantation after the heart was removed from the preservation solution was 52.3 ± 9.6, 55.6 ± 6.2, and 53.4 ± 7.3 min in groups I, II and III. Differences were not significant.

In group I massive oedema was observed when the hearts were reperfused with warm blood cardioplegia after 30 min of normothermic ischemia. Frequently, spontaneous beating
or ventricular fibrillation occurred at the beginning of reperfusion. After the second controlled reperfusion, following orthotopic heart transplantation, severe ischemic contrac
ture (‘stone heart’) was observed. None of these hearts could be weaned from CPB despite inotropic support.

In groups II and III all hearts could be weaned from CPB and none of the animals died throughout the observation period.

### 3.1. Time for weaning from cardiopulmonary bypass

In group I none of the animals could be weaned from the extracorporeal circulation. Weaning from CPB was possible after 110.8 ± 20.9 min in group II and 100.0 ± 9.6 min in group III. These differences were not significant.

### 3.2. Hemodynamics

The baseline data and hemodynamic assessment after transplantation are summarized in Table 1. In group I weaning from CPB was not possible, therefore no hemodynamic measurements after transplantation could be performed. In group II four of the nine animals required an increased preload to wean off from CPB, therefore posttransplant measurement at standardized LAP of 8 mmHg was not possible in these four experiments.

Cardiac output and mean arterial pressure were significantly decreased after transplantation as compared with the pretransplant value in group II, but not in group III. Furthermore, unpaired t-test revealed significantly higher posttransplant cardiac output in group III versus II. Heart rate was significantly increased, pulmonary vascular resistance was significantly decreased after transplant in groups II and III, as compared with the baselines.

LVSWImax and RVSWImax after transplantation were reduced significantly as compared with the preoperative value in groups II and III (Fig. 1). The recovery of posttransplantation cardiac function after 30 min of nor-

or mothermic ischemia was better in the group receiving HOE 642 (group II vs. group III): LVSWImax expressed as a percentage of the preoperative value was 27.3 ± 11.7% in group II versus 59.5 ± 32.4% in group III (P = 0.038), RVSWImax averaged 27.4 ± 20.9% versus 64.2 ± 46.5% (n.s.).

### 3.3. Heart weight.

In groups I and II heart weight was significantly increased after transplantation as compared to the pretransplant value. The increase was not significant in the HOE 642 group. Differences between the three groups were not significant (Fig. 2).

### 3.4. Enzymes

In groups II and III the levels of CK-MB and LD1 increased continuously and all differences were significant as compared with the baseline. The paired t-test revealed no significant differences between the two groups (Figs. 3 and 4).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CO (L/min)</th>
<th>Pre</th>
<th>Post</th>
<th>HR (beats/min)</th>
<th>Pre</th>
<th>Post</th>
<th>CVP (mmHg)</th>
<th>Pre</th>
<th>Post</th>
<th>MAP (mmHg)</th>
<th>Pre</th>
<th>Post</th>
<th>PAP (mmHg)</th>
<th>Pre</th>
<th>Post</th>
<th>SVR (dyne x s x cm⁻⁵)</th>
<th>Pre</th>
<th>Post</th>
<th>PVR (dyne x s x cm⁻⁵)</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>12</td>
<td>3.0 ± 0.5</td>
<td>n.m.</td>
<td></td>
<td>75 ± 8</td>
<td>n.m.</td>
<td></td>
<td>6.5 ± 1.5</td>
<td>n.m.</td>
<td></td>
<td>92 ± 13</td>
<td>n.m.</td>
<td></td>
<td>21 ± 4</td>
<td>n.m.</td>
<td></td>
<td>2194 ± 382</td>
<td>n.m.</td>
<td></td>
<td>1052 ± 300</td>
<td>n.m.</td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>9</td>
<td>3.2 ± 1.0</td>
<td>n.m.</td>
<td>2.1 ± 0.7</td>
<td>77 ± 14</td>
<td>101 ± 19</td>
<td>n.m.</td>
<td>5.1 ± 0.9</td>
<td>8.3 ± 3.5</td>
<td>n.m.</td>
<td>99 ± 12</td>
<td>68 ± 29</td>
<td>n.m.</td>
<td>21 ± 4</td>
<td>20 ± 2</td>
<td>n.m.</td>
<td>2190 ± 634</td>
<td>2198 ± 404</td>
<td>n.m.</td>
<td>490 ± 213</td>
<td>2417 ± 644</td>
<td>n.m.</td>
</tr>
<tr>
<td>Group III</td>
<td>8</td>
<td>2.9 ± 0.6</td>
<td>2.5 ± 1.2</td>
<td>n.m.</td>
<td>74 ± 16</td>
<td>123 ± 11</td>
<td>n.m.</td>
<td>5.5 ± 1.5</td>
<td>8.6 ± 1.7</td>
<td>n.m.</td>
<td>90 ± 13</td>
<td>71 ± 24</td>
<td>n.m.</td>
<td>18 ± 3</td>
<td>24 ± 8</td>
<td>n.m.</td>
<td>2417 ± 644</td>
<td>2233 ± 1007</td>
<td>n.m.</td>
<td>560 ± 311</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CO, cardiac output; HR, heart rate; CVP, central venous pressure; MAP, mean arterial pressure; PAP, mean pulmonary artery pressure; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance.

Data are given as mean ± SD.

*P < 0.05 versus pretransplant value.

n.m., No measurement possible, animals could not be weaned from CPB.
3.5. White blood cell count

Measurements revealed a reduction of white blood cells to less than 5% effected by the leucocyte filter (Fig. 5).

3.6. Postmortem macroscopic and histological examination

Macroscopic examination 2 h after the end of the CPB, revealed thickening of ventricular wall and paleness of the myocardium in all animals of groups I and II, caused by massive edema. Histological examination of explanted hearts demonstrated edema, slight infiltration of polymorphonuclear leukocytes and increased eosinophilia of the cytoplasm in severely hemodynamic compromised hearts of groups I and II but only minimal to no ischemic damage in group III.

4. Discussion

This is, to our knowledge, the first report on a successful transplantation of pig hearts after 30 min of normothermic ischemia. In contrast to most other studies on NHBD [8,9], viability of the hearts was obtained without any pretreatment of the donors. Comparison of the disappointing results in our pilot studies (group I) with the 100% graft survival in groups II and III, shows that modification of reperfusion modalities is the key for a successful outcome in this extreme model of myocardial ischemia and reperfusion.

The start of reperfusion with warm, substrate enriched blood cardioplegia after 30 min of normothermic ischemia in group I resulted in a massive myocardial oedema and ‘stone heart’ formation. None of the animals in this group could be weaned from CPB.

Therefore, we changed our strategy as following: (1) start of reperfusion after 30 min of normothermic ischemia with cold (4°C) leucocyte-depleted BCP; (2) after transplantation reperfusion was begun with tepid (20°C) leucocyte-depleted BCP at a pressure of 40 mmHg for 5 min; (3) reperfusion was continued with leucocyte-depleted non-cardioplegic blood. During this period temperature and perfusion pressure were increased stepwise to physiologic levels (37°C, arterial pressure 60 mmHg) before aortic unclamping was performed 30 min after start of reperfusion.

The change to cold instead of warm BCP at the first controlled reperfusion and to tepid BCP at the second controlled reperfusion, the introduction of a leucocyte filter, and the addition of a 30 min period of reperfusion with leucocyte-depleted non-cardioplegic blood were associated with a dramatic hemodynamic improvement of the hearts after transplantation. Since all modalities were changed simultaneously, it remains unclear, if the decreased temperature of the blood cardioplegia, the modification of the first 30 min reperfusion period after transplantation, or the leucocyte filter was responsible for the better outcome in groups II and III.

4.1. Temperature of the reperfusate

In group I controlled reperfusions were performed with

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Fig. 2. Heart weight before and after transplantation. *P < 0.05 versus pretransplant value.

Fig. 3. CK-MB values at baseline, 5, 15, 30, 60, and 120 min after the start of reperfusion. 30 min (group II), transplantation after 30 min of normothermic ischemia, 30 min HOE (group III), transplantation after 30 min of normothermic ischemia and reperfusion with HOE 642.

Fig. 4. LD1 values at baseline, 5, 15, 30, 60, and 120 min after the start of reperfusion. 30 min (group II), transplantation after 30 min of normothermic ischemia, 30 min HOE (group III), transplantation after 30 min of normothermic ischemia and reperfusion with HOE 642.
normothermic blood cardioplegia, in conformity with the concept introduced by the group of Buckberg in 1980 [10]. Numerous experimental and clinical studies have shown, that warm induction of substrate-enriched blood cardioplegia provided ‘metabolic resuscitation’ and allowed nearly complete functional and metabolic recovery in energy-depleted hearts.

According to these studies, it seemed promising to start reperfusion in our experiments with normothermic BCP. However, in the transplantation setting with long ischemic periods, storage in a 4°C solution and the absence of non-coronary collateral flow might require different protection strategies. Immediate reintroduction of 37°C warm BCP after a prolonged period (2 h) of profound myocardial hypothermia (4°C) has been inferior to the initial perfusion with tepid (20°C) cardioplegia and stepwise increase to 37°C over a 30 min period. On the other hand, the addition of a leucocyte filter to warm BCP might have resulted in a superior outcome in this experimental preparation.

4.2. Pressure of the reperfusate

The importance of the initial reperfusion pressure has been documented in several experimental [11] and clinical studies [12,13]. In groups II and III we have prolonged the period of controlled pressure perfusion (40–60 mmHg) with non-cardioplegic blood to 30 min leaving the aortic clamp in place. In addition, the volume of cardioplegia was reduced with this approach resembling the principle of the integrated myocardial protection strategy [14].

4.3. Leukocyte depletion

A lot of experimental and clinical findings provide evidence for neutrophil-mediated reperfusion injury [15]. Granulocytes contain the enzyme NADPH oxidase, can produce large quantities of superoxide and are an important source of oxygen-derived free radicals [16].

A means to halt the inflammation might allow reperfusion with granulocyte-depleted blood. Therefore, we added a leucocyte filter to our controlled reperfusion strategy which significantly reduced the number of leukocytes (Fig. 5).

4.4. Inhibition of Na+-H+-exchange

HOE 642 is a new plasma membrane NHE inhibitor. NHE is excessively activated in cardiac ischemia due to intracellular acidosis. This leads to intracellular Na⁺ overload followed by Ca²⁺ overload caused by an activation of the Na⁺-Ca²⁺ exchange. Ca²⁺ overload is responsible for the activation of proteases, lipases and phospholipases resulting in the activation of ATP utilization by many ATPases and inhibition of mitochondrial oxidative phosphorylation. This will ultimately effect cell necrosis and release of intracellular enzymes from the myocytes. During reperfusion the acidic extracellular fluid is removed and again the NHE system is excessively activated contributing to reperfusion injury. NHE inhibitors counteract the detrimental Ca²⁺ overload and therefore, prevent or reduce myocardial damage during reperfusion. In numerous in vitro and in vivo experiments the protective effect of NHE inhibitors during myocardial ischemia and reperfusion could be demonstrated. Given before myocardial ischemia NHE inhibitors prevented arrhythmias and ischemic contracture. Even when applied only during the reperfusion period, some improvement of myocardial function was observed, which however, did not reach statistical significance [17].

In conformity to this observations there was a lack of significance concerning the differences between groups II and III. Only the LVSWI max and cardiac output after transplantation were significantly increased if supplementation of blood cardioplegia with HOE 642 was performed. Mean RVSWI max was also increased in group III versus group II, but this was not significant due to the high standard deviation in both groups. There was also no significant difference between the two groups concerning increase of heart weight or myocardial enzyme release.

A problem remains that the hemodynamic performance of the hearts after transplantation is impaired. Despite 100% early graft survival in groups II and III the recovery of the hearts was incomplete. Impaired hemodynamic performance after transplantation could be caused by myocardial stunning or also by irreversible myocardial damage.

Further studies with longer observation times have to show, if adequate recovery of the myocardium is possible or if the heart muscle is damaged irreversibly. Nevertheless, further refinements of the protection strategy in this model of myocardial ischemia and reperfusion are necessary.

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References


Appendix A: Conference discussion

Dr T. Ferguson (St. Louis, MO): You stated that pig hearts have not been previously successfully transplanted, and yet you were able to accomplish this in your ‘control’ group. To what do you attribute this success?

Dr Martin: I think the main problem was myocardial protection. I refer to a work of Ferrera, who tried to use hearts from non-heart-beating donors in an isolated heart model, and he could show that after more than 10 min, when he perfused the hearts with St. Thomas solution, there was severely impaired contractility and the hearts were irreversibly damaged. And I think the main problem is the manner of reperfusion. And we all know from the Bretschneider investigations, that human hearts after 20 min of normothermic ischemia are irreversibly damaged if you perform perfusion with normal blood at normal pressure. And Buckberg could show if you modify the perfusion procedure, then you can improve the tolerance to ischemia. But we don’t know exactly when the heart is irreversibly damaged. And I think our method of controlled reperfusion with blood cardioplegia is the reason that we had better results and we could rescue these hearts after 30 min of normothermic ischemia.

Dr M. Buckburn: I would like to issue a word of caution. We have transplanted more than 30 hearts orthotopically with the aim for a rejection model to look at long-term survival. And we found that, except for one technical failure, we could wean all of them from CPB and have them stable for a period of 30 min thereafter. But the problems come much later. And of those 30, we have only half of them which survived up to 24 h, some much longer. But the real problem, and you addressed that in your last sentence in your conclusion, you will face that in the phase after weaning and thereafter in the next 12 h. And I therefore caution that it is a very optimistic view you have here at the moment from your results.

Dr Martin: Indeed, we have planned to perform these investigations as long-term experiments. We have done some pilot observations on our pigs, but these were over a 6-h period and we found that the hemodynamics remained stable over this period. But we have no experiences with weaning from respiration of these transplanted animals.

Dr A. Scheule (Tubingen, Germany): Congratulations for your interesting work. I have some questions. What do you mean by pretreatment of donor pigs? What did you actually do? Was it exsanguination? Did you do anything else?

Dr Martin: Maybe there is a bit of confusion concerning the word pretreatment. Pretreatment is the treatment of the donors with cardioprotective drugs to increase the tolerance to ischemia. This method is used by nearly all the authors who work with non-heart-beating donors, and they use steroids and prostaglandins and some other compounds. And we in our model, transplanted these hearts without donor pretreatment. The method to induce circulatory arrest was exsanguinations. And after blood pressure, zero line, we waited 30 min. And at this time the myocardial temperature averaged 32–33°C, and then we started to perform controlled reperfusion. In these 30 min we didn’t do anything else.

Dr Scheule: I would have difficulties to transfer your model into a clinical setting. Employing exsanguinations you will not have afterload during ischemia, a situation which in the future will not be present in clinical practice.

Dr Martin: I don’t understand this question. The problem was after exsanguination, if you have a circulatory arrest, you perfuse the heart and then you transplant it to an animal with a normal circulation.

Dr Scheule: My point is, you will not get non-heart-beating donors in a clinical setting in the same way your experiments are set up. You will not be able to exsanguinate them and to have a period of 30 min without


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afterload. I think the hearts will end in a different way than in your experimental model.

Dr Martin: Yes, that may be. I think at the moment there is no model we can use in the clinical practice. But there was a workshop on non-heart-beating donors held in Maanstricht in 1995, and there was discussion on some criteria for potential donors, for non-heart-beating donors. And I think, of course, this will not be a patient that comes from the road to the emergency room and we use the heart for transplantation, but there will be some other criteria. Maybe there are some donors who do not agree to an organ donation as long as the heart will beat and they agree to perform organ donation after circulatory arrest. But this is not the model of exsanguination, that’s right.

Dr B. Messmer (Aachen, Germany): It would be worth it to show electron-microscopic studies on these hearts to see how much of your tensile elements are still viable and how many are destroyed. Since you have a reduction of 60% of your tensile strength and finally also on the stroke work, you must have tremendous damage in the myocardium. Did you look electron-microscopically at these hearts or not?

Dr Martin: Yes, this is a limitation of our study. And actually we didn’t perform electron-microscopic examinations, but we intend to do it in our long-term experiments. But I think it’s not absolutely safe to differentiate between an irreversibly damaged heart and a reversibly damaged heart only by electron-microscopy. I think the most important criterion is the long-term examination.

Dr J. Vaage (Stockholm, Sweden): Related to whether the myocytes are viable or not. I think that as long as you can get off bypass with the pigs, you must have quite a lot of viable myocardium left. Since this is a very difficult model, I also think you can prove that by, for instance, testing the effect of inotropic drugs and also you can look at the release of the troponin T. So you’ll have a lot of things to do in the future, but I’m quite sure that you do have viable myocardium.