An experimental model of myocardial infarction and controlled reperfusion using a miniaturized cardiopulmonary bypass in rats

Sven Peterss, Sabina Guenther, Kristina Kellermann, Bettina Jungwirth, Ralf Lichtinghagen, Axel Haverich, Christian Hagl and Nawid Khaladj

Department of Cardiac, Thoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany
Department of Cardiac Surgery, University Hospital Munich, Ludwig-Maximilians-University, Munich, Germany
Department of Anesthesiology, Klinikum Rechts der Isar, University of Technology, Munich, Germany
Institute of Clinical Chemistry, Hannover Medical School, Hannover, Germany

* Corresponding author. Department of Cardiac Surgery, University Hospital Munich, Ludwig-Maximilians-University, Marchioninistr. 15, 81377 München, Germany. Tel: +49-89-70953460; fax: +49-89-70953465; e-mail: nawid.khaladj@med.uni-muenchen.de (N. Khaladj).

Abstract

OBJECTIVES: Different revascularization strategies for patients with acute myocardial infarction (AMI) exist. It remains unclear whether ventricular unloading using cardiopulmonary bypass (CPB) or extracorporeal life support (ECLS) has an impact on early postischaemic ventricular function. Here, we report on the results of an approach using a miniaturized CPB in a well-established animal model of AMI.

METHODS: In a randomized fashion, 30 male Wistar rats were assigned to temporary left anterior descending (LAD) ligation (30 min) followed by 180 min of reperfusion either with or without 60 min of CPB (70 ml/min, 36°C). The CPB circuit consisted of a venous reservoir, a peristaltic roller pump and a membrane oxygenator with heat exchanger. Cardiac function was measured at 60 and 120 min after reperfusion (F60, F120) using a conductance catheter.

RESULTS: The mortality rate was 37% (11/30). Thus, 19 animals could be included into the analysis (8 CPB). The mean cardiac output did not differ between the groups at F60 [63 ± 29 vs 54 ± 25 ml/min (CPB), P = 0.56] and F120 [73 ± 27 vs 53 ± 24 ml/min (CPB), P = 0.21]. During reperfusion, the mean left ventricular ejection fraction (LVEF) was stable in both the control (F60 37 ± 5% vs F120 33 ± 8%, P = 0.42) and the CPB groups (F60 52 ± 11% vs F120 51 ± 13%, P = 0.71). CPB animals had a significantly better LVEF after reperfusion (F60 P = 0.007, F120 P = 0.01).

CONCLUSIONS: In this animal model of AMI, the establishment of CPB resulted in a significantly better LVEF in comparison with conventional reperfusion only. This beneficial effect may have an impact on revascularization strategies and timing in patients presenting with AMI in the future.

Keywords: Myocardial infarction • Ventricular unloading • Controlled reperfusion • Rat model • Cardiopulmonary bypass • Extracorporeal life support

INTRODUCTION

Cardiogenic shock caused by acute myocardial infarction (AMI) is associated with high mortality. Temporary circulatory support by intra-aortic balloon counter pulsation (IABP) or temporary implantable devices was not able to reduce mortality rates [1, 2]. Percutaneous extracorporeal life support (ECLS) is a therapeutic option for refractory cardiogenic shock due to various underlying pathologies [3].

ECLS therapy in patients suffering from severe progressive cardiogenic shock after successful revascularization by percutaneous coronary intervention leads to beneficial results in comparison to IABP therapy only [4, 5]. This might be due to ventricular unloading and controlled reperfusion [6].

In experimental set-ups, ventricular unloading via atrio-femoral artery bypass preceding coronary reperfusion after prolonged ischaemia led to reduced myocardial damage in swine [7]. To verify the beneficial effects of controlled reperfusion, we modified an established rodent animal model of myocardial infarction using miniaturized cardiopulmonary bypass (CPB) systems [8–10].

MATERIALS AND METHODS

Technology

The CPB single-circuit system for small animals (Martin Humbs, Engineering and Precision Mechanics, Valley, Germany) is a closed extracorporeal circuit consisting of a roller pump, a venous reservoir

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In this experimental study, a well-known rodent model of myocardial infarction was modified by additional use of CPB. The study was conducted at Hannover Medical School. Thirty adult male Wistar rats (weight 400–500 g) were randomized into two groups. In all animals, temporary anterior myocardial ischaemia was induced by LAD artery ligation for 30 min. Subsequently, 60 min of normothermic reperfusion either with or without CPB followed. CPB was established 5 min prior to reperfusion. All animals were observed for 2 h after conventional reperfusion or reperfusion using CPB including haemodynamic measurements via a conductance catheter. At the end of the experiment, blood and tissue samples were harvested for further analyses. All animals received human care in compliance with the guidelines ‘Principles of Laboratory Animal Care’ formulated by the National Society for Medical Research and the ‘Guide for the Care and Use of Laboratory Animals’ published by the National Institute of Health (NIH Publication No. 88–23, received 1996). The protocols for all experiments were approved by the Hannover Medical School Institutional Animal Care and Use Committee as well as by the Federal State of Lower Saxony, Germany.

### Anaesthesia, perioperative management and myocardial infarction

After anaesthetization with isoflurane 2.5%, pentobarbital 60 mg/kg (intraperitoneal injection) and atropine 50 µg (s.c.), the rats received a tracheostomy with a 16 G polyethylene tube and were mechanically ventilated with a respiratory frequency of 60/min, an end-inspiratory pressure of 11.9–20.6 cmH₂O and an oxygen flow of 0.95–1.6 l/min (PaCO₂ 32–45 mmHg) (KTR-5, Hugo Sachs, March-Hugstetten, Germany). Anaesthesia was maintained using 1.0% isoflurane, and 150 IU heparin (s.c.) was administered. To maintain normothermia, the animals were positioned supine on a heating pad. Continuous rectal temperature measurement and electrocardiography (ECG) monitoring using limb leads were implemented.

After preparation of the tail artery, it was cannulated with a 20 G polyethylene tube, and used as arterial inflow cannula of the CPB and for periodical arterial blood gas analyses [10]. For venous drainage, a silicone multiple-stage cannula was inserted into the right jugular vein. The tip was positioned within the inferior vena cava. The left jugular vein was cannulated with a small catheter to infuse fluids and drugs. Via the left carotid artery, a 2-F conductance catheter was inserted into the ascending aorta. Afterwards, median sternotomy, resection of the thymus and opening of the pericardium were performed. Systemic heparinization followed by intravenous application of 500 IU/kg.

To initiate myocardial ischaemia, an 8-0 polypropylene suture with a soft pledget (polytetrafluoroethylene) was purged around the LAD branch of the left coronary artery and tightened with a tourniquet to achieve temporal ligation. Myocardial ischaemia was verified by ECG changes. After 30 min of myocardial ischaemia, the tourniquet was released and 60 min of reperfusion either with or without CPB followed. Afterwards, all animals were observed for 2 additional hours.

Heparin was antagonized in a 1 : 0.75 fashion. Ventilation and metabolic parameters were regularly checked by blood gas analyses. Oxygen flow and end-inspiratory pressure were adjusted according to PaO₂, PaCO₂ and base excess (BE). A BE < -3 was compensated with 0.1 ml of sodium bicarbonate per 1 BE. Glucose levels were maintained above 6 mmol/l.

### Extracorporeal circulation and postoperative management

CPB was initiated after 25 min of myocardial ischaemia. The priming solution of the CPB circuit was 8 ml in volume including hydroxyethyl starch (HES) 6% and 50 IU heparin. The nominal flow of the normothermic CPB was calculated as with 70–80 ml/min and the mean blood pressure was adjusted to at least 50 mmHg. No catecholamine support was used while on CPB. The animals were additionally ventilated to avoid atelectasis. Metabolic parameters were monitored online by optical fluorescence- and reflectance-based in-line measurements (CDI™ Blood Parameter Monitoring System 500, Terumo®, Eschborn, Germany) and adjusted to normal ranges. After 60 min of reperfusion, the animals were weaned from CPB and heparin was antagonized (1 : 0.75). The circuit tube volume was centrifuged; 1 ml of calcium and 1 ml of sodium bicarbonate solution were added. The resulting concentrated red blood cells were retransfused at 2–5 ml/h.

### Haemodynamic measurements and area-at-risk analysis

The 2-F conductance catheter (MIL-SPR-838 (Pressure/Volume Micro Tip Catheter for Rats, 2.0-F, 9-mm conductance electrode segment), Millar Instruments, FML, Seeheim, Germany) was inserted via the left carotid artery and positioned within the left ventricle. Steady-state pressure-volume loops (PVLs) were recorded to obtain volumetric and functional parameters; parallel conductance of the myocardium was measured via injection of hypertonic saline into the left jugular catheter and subtracted [11]. To obtain functional parameters being independent of preload, we temporarily occluded the inferior cava vein and recorded the corresponding PVLs. All data were collected and analysed using MPVS-Ultra and PowerLab. A calibration series was performed to adjust the results according to the haematocrit [12].

After 2 h of observation of the anaesthetized animals, all animals were sacrificed. In advance, blood samples were taken for further analyses.

The LAD was retied using the tourniquet and Coomassie blue (2%) stain was injected directly via the ascending aorta to mark the area at risk. The heart was cut into 1.5-mm slices and immersed in triphenyltetrazolium chloride at 37°C for 10 min. The slices were scanned and the area at risk was analysed planimetrically in...
Experimental protocol

- Surgical preparation, systemic heparinization, baseline data collection
- 30 min of temporary myocardial infarction, collection of ischaemia data after 25 min (I25)
- 60 min of reperfusion, collection of reperfusion parameters after 60 min (R60)
- 2-h follow-up period with haemodynamic measurements using the conductance catheter technique (F60, F120)
- Harvesting blood and tissue samples for further analyses and determination of the area at risk.

Laboratory parameters

Blood samples were immediately centrifuged and the supernatant harvested and stored at −20°C for further analysis in the Institute of clinical chemistry. Myeloperoxidase (MPO), copeptin and creatinkinase (CK) were detected using commercially available kits (Hölzel Diagnostika, Cologne, Germany).

Statistics

Categorical variables are given as numbers and percentages. Data concerning continuous variables are expressed as mean ± standard deviation. Statistical analysis for group comparison was performed using the t-test. IBM SPSS Statistics software, Version 20 was used for statistical analysis. A P-value of <0.05 was considered statistically significant.

RESULTS

Mortality

The mortality rate was 37% (11/30). Seven animals in the CPB group died; 4 in the control group.

In 4 cases, the animals died due to technical reasons (13%, 2 control animals, 2 CPB animals). Seven did not survive the myocardial infarction procedure (20%, 2 control animals, 5 CPB animals). Thus, 19 animals could be included into the analysis (11 control animals, 8 CPB animals).

Perioperative data

Perioperative data are depicted in Table 1. Laboratory parameters obtained solely at the end of the experiment are presented in Table 2. To maintain sufficient blood pressure levels (±50 mmHg) after weaning from CPB, vasopressors frequently had to be used.

Cardiopulmonary bypass data/flow

Flow parameters were stable during the experiment (Fig. 1). Neither catecholamines nor blood products were administered while on CPB.

<table>
<thead>
<tr>
<th>Table 1: Haemodynamic and metabolic parameters</th>
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<tr>
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<tr>
<td>Heart rate (bpm)</td>
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<tr>
<td>MAP (mmHg)</td>
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<tr>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>pH</td>
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<tr>
<td>Haemoglobin (g/dl)</td>
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<td>Haematocrit (%)</td>
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<td>Lactate (mmol/l)</td>
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| I25: ischaemia 25 min; R60: reperfusion 60 min; F60: follow-up 60/120 min; CPB: cardiopulmonary bypass; MAP: mean arterial blood pressure.
Conductance catheter measurements

Cardiac output (CO) as well as left ventricular ejection fraction (LVEF) were calculated. The mean CO did not differ between the two groups at R120 ($P = 0.56$) and R180 ($P = 0.21$) (Fig. 2). CPB animals had a significantly better LVEF during reperfusion in comparison with control animals ($R120 P = 0.007$, $R180 P = 0.01$) (Fig. 3). Within each group, the mean LVEF did not change ($F60 37 \pm 5\%$ vs $F120 33 \pm 8\%$, $P = 0.42$ for control animals and $F60 52 \pm 11\%$ vs $F120 51 \pm 13\%$, $P = 0.71$ for CPB animals, respectively).

Area-at-risk analysis

In CPB animals, the area at risk was significantly larger ($P = 0.02$) (Fig. 4).

DISCUSSION

In rat models, the overall mortality rate after coronary occlusion ranges from 13 to 65%; 44% of male and 17% of female rats die immediately [13, 14]. Technical problems while establishing CPB occur in up to 10% [10].

In our series, 37% of the animals did not survive until the end of the experiment due to various reasons. Altogether, the mortality rate within our series is thus comparable with those described earlier.

A peculiarity of this model is the huge extension of the area at risk despite standardized LAD occlusion [15]. Rats do not have a true circumflex artery; the septal branch originates from the ostium. Thus, this model results in infarction of the left ventricular free wall without, however, involving the interventricular septum [16, 17]. To determine the area at risk, measurement of the ischemic area, e.g. by planimetry, is recommended [18].

The ischemic area was significantly larger in CPB animals. In general, an infarction size below 30% does not result in an impaired haemodynamic situation, whereas infarction sizes between 31 and 46% lead to a reduced pressure-generating capacity of the myocardium [19]. On top, after weaning from CPB, a drop in blood pressure levels may occur [9]. Both, the larger ischaemic area as well as blood pressure fluctuations during and after the weaning procedure may therefore account for a need of vasopressors in CPB animals. In experimental set-ups, moderate systemic hypotension during reperfusion was associated with an increased myocardial infarction size and decreased coronary blood flow [20].

Haematocrit levels were significantly lower in the CPB group due to haemodilution; no blood products were administered to avoid the adverse effects of transfusion. Varying haematocrit levels were considered during conductance catheter measurements [9].

### Table 2: Laboratory parameters

<table>
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<tr>
<th>Parameters</th>
<th>Control</th>
<th>CPB</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Myeloperoxidase (pg/ml)</td>
<td>78 183 ± 17 000</td>
<td>46 501 ± 4883</td>
<td>0.002</td>
</tr>
<tr>
<td>Creatinkinase (U/l)</td>
<td>51 ± 22</td>
<td>34 ± 10</td>
<td>0.04</td>
</tr>
<tr>
<td>Copeptin (pg/ml)</td>
<td>59 ± 91</td>
<td>236 ± 57</td>
<td>0.002</td>
</tr>
<tr>
<td>Troponin T (pg/ml)</td>
<td>260 ± 51</td>
<td>440 ± 121</td>
<td>0.005</td>
</tr>
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CPB: cardiopulmonary bypass.
Negative rheological effects of increasing plasma viscosity due to administration of HES tend to be offset by the concomitant reduction of the haematocrit [21]. Thus, myocardial perfusion and oxygenation in CPB animals might not be compromised. However, haematocrit levels influence the oxygen transport capacity of the blood and the effects of concomitant haemodilution due to the priming volume of the CPB, potential blood loss and the effect of myocardial ischaemia itself have not been investigated in the current set-up.

We saw a significantly better postischaemic LVEF in CPB animals compared with the control group.

Postischaemic mean Troponin T and copeptin were significantly higher in CPB animals, whereas CK and myeloperoxidase were lower. Copeptin seems to quantify the individual endogenous stress level and is a marker of mortality in patients with AMI. In contrast, MPO is a useful parameter predicting long-term survival and LVEF after AMI. Furthermore, both parameters help to diagnose AMI at early stages [22]. However, measuring these parameters immediately after the ischaemic phase might be too early to have a prognostic impact. Furthermore, the exact influence of CPB itself on both time course and dimensions of the parameters obtained has not been elucidated so far. Further studies are warranted to address this issue.

Taking into account that the area at risk was significantly larger in the CPB group and that the size of this area correlates with a potential worse outcome and a decreased LVEF, the benefit of CPB might even be underestimated in this trial.

A possible explanation of our findings has been published in the 1990s. Controlled reperfusion in patients with AMI was associated with a better outcome regarding mortality and recovery of regional wall motion disorders despite longer periods of ischaemia [6]. In more recent studies from our group, mortality in patients with ST-elevation myocardial infarction undergoing immediate surgical revascularization was substantially high but lower than the mortality rates in the shock trials [23]. This might be due to the fact that total revascularization was performed instead of only revascularizing the culprit lesion, which might also prevent progression of the cardiogenic shock. In addition to cold blood cardioplegia and controlled reperfusion, IABP was frequently used. Using a prospective treatment protocol for patients with AMI, mortality rates could be further reduced in comparison with our previous experience [24]. However, randomized trials do not exist till now to prove this hypothesis. The benefit of IABP in cardiogenic shock is under discussion and ventricular unloading by CPB/femoral arterial line ECLS resulted in a better outcome compared with IABP treatment in shock patients undergoing PCI [2, 5].

In an experimental set-up, mechanical unloading of the left ventricle prior to coronary reperfusion by a left atrial-femoral bypass with 50% of the initial CO for 120 min reduced left ventricular wall stress and myocardial infarction size despite longer ischaemia [7]. This is in line with earlier results of retrospective patient data analyses [6].

Even though afterload reduction and thus mechanical unloading by intra-aortic counter pulsation was able to reduce the infarction size in animals, randomized trials failed to prove this effect in humans [2, 25]. So far, the reason for this discrepancy remains unclear.

All studies besides the one described above used various animal models or ways of ventricular unloading. However, in all cases, direct unloading of the ventricle was performed.

To the best of our knowledge, our study is the first experimental trial showing beneficial effects of ventricular unloading by CPB/ECLS. As in the majority of other studies, the exact mechanism of this effect remains unclear. Providing full CO prior to coronary reperfusion and therefore maintaining systemic and coronary perfusion might reduce reperfusion injury. Another beneficial effect could be a reduced myocardial oxygen demand during reperfusion [7].

Limitations

This model goes along with a substantial variation of the size of the infarction despite standardized occlusion of the LAD. The impact of possible CPB-associated side effects such as activation of the inflammatory system, haemodilution, blood pressure drops and variability during reperfusion remain unclear. Data indicating myocardial oxygen consumption, coronary flow or myocardial wall distress are missing. No preoperative CO measurements were obtained due to the fear of malignant arrhythmias prior to ischaemia. Even though the data presented seem to clearly indicate a beneficial effect of ventricular unloading, the concept yet has to be proved pathophysiologically. Afterwards, verification of beneficial effects is necessary.

Whether initial superiority of LVEF persists cannot be concluded from this acute-phase animal model.

CONCLUSIONS

In this animal model of AMI, the use of CPB prior to reperfusion to achieve ventricular unloading led to a significantly better LVEF in comparison with revascularization therapy only. The effect seen in our and other experimental as well as clinical studies seems to improve ventricular function and survival especially in severe cases of AMI complicated by cardiogenic shock. This beneficial effect may have an impact on timing and revascularization strategies in patients presenting with AMI, including surgical approaches as well as ventricular unloading by means of ECLS or other devices.

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Conflict of interest: none declared.

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