Effects of pulmonary artery perfusion on gas exchange and alveolar matrix metalloproteinases after cardiopulmonary bypass in a swine model

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Applied Cardiopulmonary Pathophysiology 13: 123-129, 2009

Keywords: matrix metalloproteinase, acute lung injury, cardiopulmonary bypass, hypothermic lung perfusion

Abstract

Background: The lung is the least protected organ during cardiopulmonary bypass (CPB). The present pilot-study was designed to determine the effects of the infusion of cooled, oxygenated blood into the pulmonary artery as hypothermic lung perfusion (HLP) on gas exchange and inflammation after CPB in an animal model.
Methods: Pigs were randomly assigned to a control group (n=6) undergoing CPB with moderate hypothermia and 60 minutes of cardioplegic cardiac arrest or an intervention group being additionally treated with 45 minutes of HLP during aortic crossclamping. The alveolo-arterial O2-gradient ($AaDO_2$), the concentrations of the metalloproteinases (MMP) 2 and 9 in bronchoalveolar lavage (BAL), and the relative count of nucleophils (RCN) were determined.
Results: $AaDO_2$ increased in both groups but was lower after CPB in the HLP in comparison with the control group (384 ± 146 vs 542 ± 75 after 120 minutes; 377 ± 172 vs 541 ± 73 after 180 minutes; $p<0.05$). The RCN decreased in the HLP group compared to control immediately after CPB ($p<0.05$) while the MMPs 2 and 9 increased in both groups without any difference after 180 minutes.
Conclusions: This suggests that HLP may improve pulmonary gas exchange and that this may – at least in part – be related to a decrease in post-CPB pulmonary inflammation.

Introduction

Cardiopulmonary bypass (CPB) triggers a systemic pro- and anti-inflammatory cascade that may induce postoperative complications including multiple organ dysfunction [1]. A number of events can initiate the systemic inflammatory response during cardiac surgery, including blood contact with the artificial surface of the CPB, endotoxemia, or the development of ischemia or reperfusion injury [2]. Postoperative acute lung injury (ALI) contributes to the morbidity and mortality after CPB, especially in patients with preexisting lung disease [3]. A key role in the development of ALI after CPB and ARDS is the activation of neutrophils and the following induction of proteinases and oxygen radical species [4]. The collagenatic matrix metalloproteinases MMP 2 and 9 are type IV collagenases which are able to destruct the basement membrane of the lung as a molecular structure. MMP 2 (72 kDa) is produced by alveolar cells, endothelial cells and fibroblasts, whereas MMP 9 (92 kDa) is mainly produced by cells of the inflammatory cascade like neutrophils. Elevated matrix metalloproteinases 2 and 9 in the bronchoalveolar lavage (BAL) indicate acute lung injury after CPB [5-7].

Respiratory dysfunction occurs in virtually all patients undergoing CPB [8], and it has been recognized that CPB is associated with pulmonary ischemic-reperfusion injury [9]. However, as far as preventing tissue ischemia during CPB is concerned, the lung remains one of the least protected organs. Ischemia of the lung during CPB may cause ultrastructural changes of lung parenchyma by diminished bronchial arterial blood flow [10]. This pulmonary injury may be re-

duced by controlled pulmonary perfusion [11]. Moreover, data from the field of lung transplantation cleary indicate that the application of cooled, oxygenated blood is beneficial for the preservation of lung transplants [2, 12] which suffer ischemia/reperfusion injury during storage and transplantation.

We hypothesized that cooling of the lungs by hypothermic pulmonary artery perfusion with oxygenated blood during moderately hypothermic CPB would have a protective effect and can attentuate the deterioration of lung function as well as inflammatory reaction monitored by markers like MMP-2 and 9 after CPB.

Material and method

Animals

The experimental protocols used in the present study were approved by the appropriate governmental authority. All animals received humane care in accordance with the „Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1985).

Anaesthesia

17 pigs (mean weight: 32 kg) were premedicated with intramuscular ketamine (15 mg * kg^{-1} body-weight [bw]), 2 mg * kg^{-1} bw xylazine, and 0.5 mg atropine. General anaesthesia was induced by an intravenous bolus injection of sufentanil (0.3 µg * kg^{-1} bw) plus propofol (3 mg * kg^{-1} bw) plus pancuronium (0.1 mg * kg^{-1} bw), and was maintained with intravenous sufentanil (1 µg * kg^{-1} bw * h^{-1}) and propofol (3-5 mg * kg^{-1} bw * h^{-1}). The pigs were intubated with a disposable endotracheal tube (ID 7.0 mm, Brand, Mallinckrodt, Hennef/Sieg, Germany) via tracheotomy. The volume-controlled mechanical ventilation (FiO_2 = 1.0, PEEP = 5 mbar, I:E ratio = 1:1.7) parameters (tidal volume 8 - 10 ml * kg^{-1} bw; ventilation rate 10-15 min^{-1}) were adapted to maintain physiological values of pH and pCO_2. The instrumentation included intravascular catheters to monitor systemic, pulmonary arterial, pulmonary capillary wedge and central venous pressures. Arterial blood gas samples were used for the estimation of pulmonary gas exchange. Body temperature was measured rectally and kept constant using an external electrical heater.

Cardiopulmonary bypass

A median thoracotomy was performed and the cannulation for cardiopulmonary bypass (CPB) was prepared. 300 IU /kg heparin was given and the activated clotting time [ACT] monitored every 30 minutes (3000 IU heparin were administered again, if needed, to maintain an ACT > 400 s). The extracorporal circuit was primed with fresh allogenic blood. After canulating the ascending aorta (arterial cannula 6 mm, angled) and the right atrium (customized 0.5 inch punnet), CPB was started (membrane oxygenator: Hilite, Medos, Stolberg, Germany; microfilter: DII AF-1040, Baxter, Unterschleissheim, Germany). The CPB was conducted with mild hypothermia (32°C rectal temperature) according to standard techniques. Mean arterial blood pressure was kept between 60 and 80 mmHg, mainly by changing the flow (>2.4l*min^{-1}*m^{-2} body surface area). The aorta was clamped and cardiac arrest was induced by cold (4°C) crystalloid (St.Thomas') antegrade cardioplegia and maintained for 60 minutes by repetitive application of cardioplegia every 20 minutes. Following rewarming, the animals were weaned from CPB. Low-dose epinephrine (0.1µg/kg/min) was given to all animals for 60 minutes. The observation period ended 180 minutes after weaning from CPB and the animals were terminated in deep anaesthesia.

Experimental protocol of lung protection

Hypothermic lung perfusion vs. control

The pigs were randomized in a 2:1 fashion into two groups: 11 pigs for the hypothermic lung perfusion group (HLP) and 6 pigs for the control group (C). In group HLP, lung perfusion (200 ml/min) was started after the pulmonary artery had been cannulated and 100% of the calculated systemic pump flow had been achieved. The pulmonary artery was cannulated with an angled 4.0 arterial cannula (Sherwood Medical GmbH, Sulzbach, Germany). For the pulmonary artery perfusion arterial blood from the CPB was separated and cooled down to 5° C by a separate heat exchanger (D270 P, Dideco, Mirandola, Italy). The lung perfusion line included a 40µm arterial filter (D733, Dideco). Lung temperature decreased down to approximately 18 ° C during lung perfusion. In the control group the temperature drop was less pronounced only due to the mild hypothermia (32° C) of CPB [13].

Time points of measurements

In both groups hemodynamic and respiratory parameters were measured and blood samples were drawn immediately before preparation of CPB as baseline (T0), and at time points 5(T5), 30 (T30), 60 (T60), 90 (T90), 120 (T120), 150 (T150) and 180 (T180) minutes after CPB weaning.

Measured variables

Hemodynamic monitoring was performed with a Sirecust monitor (Siemens Medical, Erlangen, Germany). Compliance and mean airway pressure were determined by the airway monitor PM 8050 (Dräger Medical, Luebeck, Germany).

Alveolar arterial gradient of oxygen tension ($AaDO_2$)

The $AaDO_2$ is the difference of the partial pressures of alveolar (pAO_2) and arterial oxygen (paO_2). PAO_2 was calculated as follows [14]:

$PAO_2 = FiO_2*((BP-P_{H2O}) - (PaCO_2/RQ)*(1-FiO_2$
$(1-RQ))$,

where FiO_2 is the fractional concentration of oxygen in the inspired air, BP the barometric pressure, P_{H2O} the saturated vapour pressure, $PaCO_2$ the arterial carbon dioxide tension pressure and RQ the respiratory quotient. PaO_2 and $PaCO_2$ were measured by a blood gas analyzer (ABL, Radiometer, Copenhagen, Denmark).

Venous admixture Qs/Qt

The venous admixture was calculated to the formula:
Qs/Qt = (CcO2-CaO2)/(CcO2-CvO2), where Qs is the shunt blood and Qt the cardiac output. CcO2, CaO2 and CvO2 are the oxygen content in end capillary, arterial and mixed venous blood. The oxygen content CxO2 is calculated by the equotation:
CxO2 = 0.0031 * pxO2 + 1.39 * Hb concentration * SxO2, where x are arterial or mixed-venous, respectively.

Relative neutrophil counts ratio

Blood was collected subjected to Coulter Counter (Electronic LTD, Luton, GB) analysis of total leukocyte concentrations and subsequent manual leukocyte differential count to determine the numbers of neutrophils. The ratios between the numbers of neutrophils at each time point (T0 – T180) and baseline (T0) were calculated to describe the relative change as follows:

Ratio T(x) = Concentration of neutrophils at Tx/ Concentration of neutrophil at T0.

Tx were the time points T0, T5, T60, T120 and T180.

Matrix metalloproteinases MMP-2 and -9

For collecting the bronchoalveolar fluid samples at T0, T5 and T180 the bronchial system was blindly intubated with a suction catheter. Immediately after instillation of a 50 ml NaCl 0.9% bronchoalveolar fluid was gently withdrawn parallel to the collection of blood samples. The samples were spun to separate the cells from the supernatant (3000 U/min for 20 min), which was frozen and stored at -70°C for further analysis. For the evaluation of the MMP we further processed with a SDS gel electrophoresis to separate the bands for further negative staining (Coomasie brilliant blue dye solution) and analysis (Image analyzer Easy Rev. 413, Herolab, Molekulare Trenntechnik, Wiesloch, Germany). Further details were described elsewhere [5].

Statistical analysis

The data for hemodynamic and respiratory parameters are presented as mean and standard deviation of the mean as appropiate, and the student t-test for paired and unpaired samples were done. The data for the metalloproteinases and the relative leukocytes counts presented were not assumed to be normally distributed, and non-parametric procedures were chosen for statistical difference.Therefore these values are presented as median, 25% and 75% percentile. Due to the explorative study design, p-values were not adjusted for multiple comparisons and were only used for descriptive purposes. Wherever a difference between the time points of the same group or between groups at the same time point is stated, the corresponding test statistic produced a p-value of less than 0.05.

Differences in location of more than two dependent samples were determined by the Friedman test, followed by Wilcoxons signed-rank test comparing the baseline with the following time points. Differences between the groups were analysed using the Mann-Whitney U-test. Data were analyzed with the "Statistical Package for Social Sciences" (SPSS) [Chicago, II., USA].

Results

There were no signs of left or right heart decompensation as PCWP and pulmonary pressures did not change significantly during the study (table 1).

Compliance decreased and mean airway pressure increased after CPB for both groups ($p<0.05$) but there were no differences between them (table 1). The venous admixture increased only for control group at T5 compared to baseline, but there was no intergroup difference. AaDO2 increased significantly in both groups after CPB; however this increase was significantly less prominent for HLP at T120 and T180 ($p<0.05$) (see figure 1).

The ratio of neurophils in the BAL decreased immediately after CPB at T5 for group HLP vs control ($p<0.05$); the ratio was decreased in both groups at T180 (see fig.2). The activity of MMP-2 in the BAL appeared to increase after CPB in both groups; the increase appeared to be slower but more pronounced in the control-group (see fig.3). A similar pattern was observed for MMP-9, but there were no group differences (see fig.4).

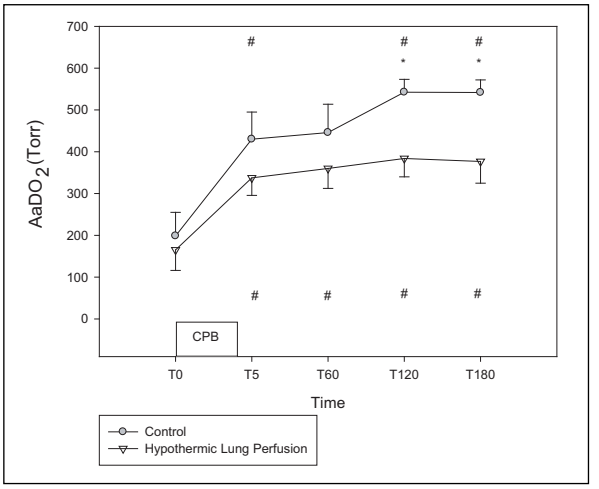
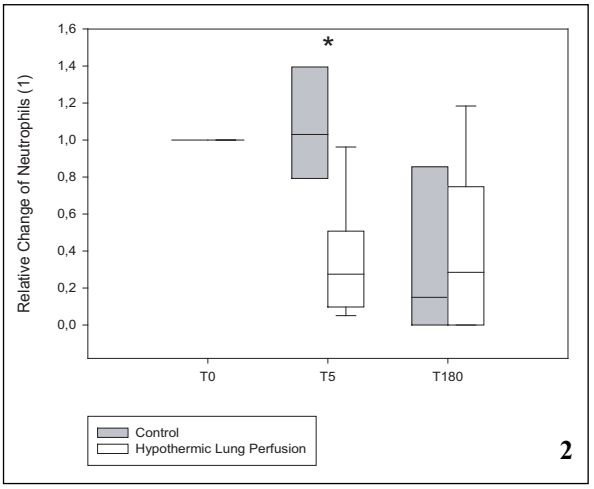


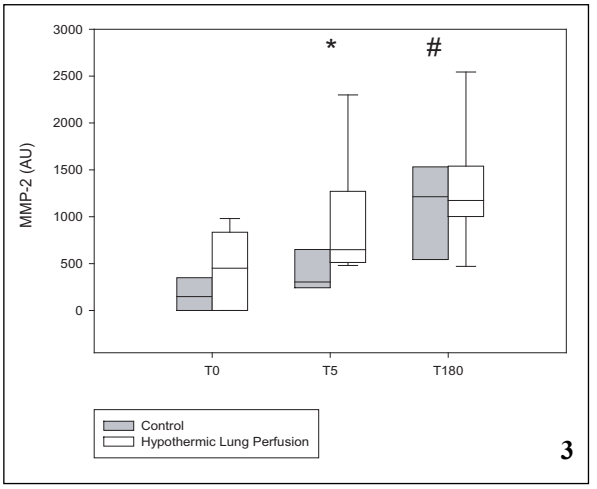
Figure 1:
 # : p < 0.05 difference to baseline data at T0,
** : p < 0.05 intergroup difference.*
T0: Time point before preparation of CPB,
T5, T60, T120 and 180: Time points at 5, 60, 120 and 180 minutes after CPB.
AaDo2, Alveolo-arterial difference; CPB, Cardiopulmonary bypass

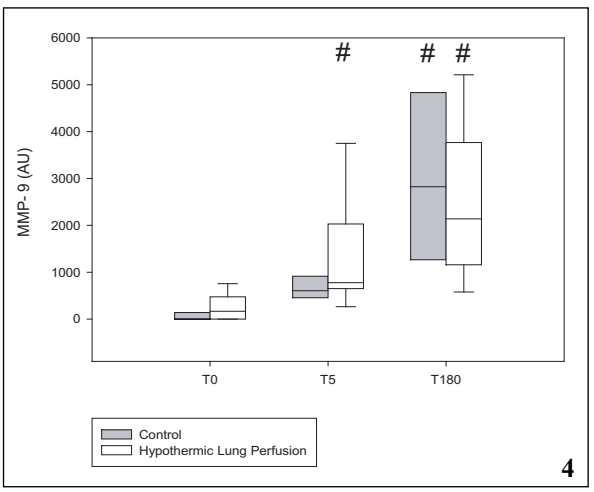
Table 1. Hemodynamic and pulmonary changes in two groups

Parameter	Group	T0	T5	T60	T120	T180
PCWP (mmHg)	HLP	8±1	10±1	8±1	8±1	8±1
	C	9±1	9±1	9±1	9±1	8±1
PaP syst (mmHg)	HLP	24±6	27±2	26±2	23±2	25±3
	C	21±2	26±1	25±2	25±3	24±1
Pa mean (mmHg)	HLP	18±4	20±2	17±1	13±1	16±2
	C	15±2	21±1	18±2	19±3	17±2
Pa diast (mmHg)	HLP	12±3	15±2	12±1	12±2	10±2
	C	12±2	16±1	14±2	15±3	12±2
Comp (ml/cm H_2O)	HLP	49±5	36±4 a	32±2 a	29±3 a	26±3 a
	C	59±7	33±3 a	32±5 a	27±3 a	21±2 a
AWPm (mbar)	HLP	4±0	4±0	5±1	5±0	5±0 a
	C	5±1	5±1	5±1	5±1 a	6±1 a
Qs/Qt (%)	HLP	4.1±1.0	5.4±0.7	6.2±0.7	6.7±1.1	6.5±1.2
	C	4.6±1.6	9.1±3.0 a	9.9±4.1	9.3±3.2	4.7±0.6

a : $p < 0.05$ difference to baseline data at T0,
T 0: Time point before preparation of CPB,
T5, 60, 120 and 180: Time points at 5, 60, 120 and 180 minutes after CPB.
$AaDO_2$, Alveolar-to-arterial gradient of oxygen tension; AWPm, mean airway pressure; Comp, Compliance; diast, diastolic; Pa, pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; Qs/Qt, venous admixture; syst, systolic; HLP, hypothermic lung perfusion; C, control.







Figures 2-4.
 # : p < 0.05 difference to baseline data at T0,
** : p < 0.05 intergroup difference.*
T0: Time point before preparation of CPB,
T5 and 180: Time points at 5 and 180 minutes after CPB.
MMP, metalloproteinase; AU, arbitrary unit.

Discussion

We demonstrated that hypothermic pulmonary artery perfusion with oxygenated blood improved $AaDO_2$ under experimental conditions in pigs. We could demonstrate that treatment with HLP did not avoid an inflammatory reaction as both MMP 2 and MMP 9 had risen. Nevertheless, we found a reduced relative count of neutrophils immediately after CPB with HLP. An increase of $AaDO_2$ can be caused by ventilation/perfusion mismatch, right to left intrapulmonary or intracardial shunting. In our setting, an intracardiac shunt is highly unlikely. In our opinion the increased $AaDO_2$ is most likely caused by swelling of the epithelial cells and of the subepithelial membrane after CPB and/or by an atelectasis. The observed increase of the MMPs might theoretically also be caused by myocardial failure [15, 16]. However, a right or left heart decompensation is highly unlikely as there were no hemodynamic signs of myocardial failure. In addition, myocardial failure was ambly tried to prevent by prophylactic infusion of epinephrine during weaning from CPB and in the first hour thereafter.

The type IV collagenatic MMP-2 and -9 can destroy the molecular structure of the basement membrane of the lung. After infection or injury, interleukin 1 and tumor necrosis factor Alpha stimulate the production of these MMPs. Under physiologic conditions, a group of specific tissue inhibitors (TIMP) controls the synthesis and activity of MMPs [17, 18]. Any mismatch in favor of more MMP activity therefore can lead to the destruction of the basement membranes of the lung. Of notice, the balance between the MMPs and the TIMPs was also observed to be disturbed by CPB-related injury to the lung [5, 19]. MMPs in bronchoalveolar lavage fluid were found to be elevated in

patients with ARDS [20]. A nitric oxide scavenger is able to reduce the MMP-2 and -9 activities after CPB in different organs including the lungs as demonstrated by Mayers et al. in an experimental study [21]. Carney could demonstrate that a chemically modified tetracycline is able to inhibit enzymes such as elastase and MMPs, which are both responsible for the destruction of the basement membrane [22]. Future investigations therefore should explore the protective effects of therapeutic agents and procedures in relation to the activity of MMPs and TIMPs as their counterparts.

Post CPB respiratory dysfunction may lead to life threatening acute lung injury or adult respiratory distress syndrome, encouraged by further risk factors as pre-existing lung disease or circulatory arrest. In a different model, where the lung artery is occluded and reperfused again, an impairment of the bronchial perfusion promotes an increased capillary leakage [23]. An explanation for our results regarding $AaDO_2$ may be that this damage of the lungs is lessened by protective effects of HLP during CPB. While CPB generates an ischemia of the lungs whose extent is difficult to judge, HLP with cooled and oxygenated blood supplies the lung and further decreases its oxygen consumption by cooling it down. Initial hypothermic lung perfusion was used in lung surgery to protect the transplant [24]. Sievers et al. demonstrated in a clinical investigation that HLP had a protective effect on gas exchange [25]. In their study, the HLP with cooled oxygenated blood was claimed to result in better gas exchange (as shown by diminishing an increase of $AaDO_2$ post CPB) and less lung inflammation (as shown by diminished concentrations of elastase alpha 1 proteinase inhibitor complex and alpha 2 macroglobulin in the bronchoalveolar lavage).

Beside the use of the hypothermic lung perfusion for lung protection further therapeutic strategies have been investigated under experimental and clinical conditions. During lung procurement and storage, flushing the lung vessels with potassium-rich solutions beneficially influences $AaDO_2$ [26]. Other experimental procedures to protect the lung and the gas exchange after transplantation are ischemic preconditioning [27], which is transduced by the adenosine receptors of the pulmonary vessels. Further concepts were the use of taurin [28] or aprotinin [29, 30] as an antioxidant. Whether these procedures are of benefit in non-transplant settings is unknown. As hyothesised by our group and substantiated by this experiment and by clinical data [25], the inflammatory reaction during CPB can be reduced by pulmonary perfusion. However, pulsatile flow may even increase this effect [31].

The neutrophils play a key role in triggering the inflammatory reaction in the lung. In our study, the observed decrease of relative neutrophil count immediately after CPB with HLP can be interpreted as a diminished inflammatory response. However, it is unclear whether the neutrophil reaction is the cause or the response of further lung injury [7, 32].

This study has several limitations. One is the small number of animals and the resulting low statistical power. A more detailed investigation of hemodynamic and inflammatory parameters, especially of neutrophil and endothelial activation, should focus on the pathway and control of inflammation more precisely. The results have also to be cautiously regarded as signs of lung injury are more often noticed in pigs [33].

Our investigation suggests that hypothermic lung perfusion during CPB seems to have a protective effect on pulmonary gas exchange but is not able to suppress the increase of the inflammatory markers investigated. This contradiction should be investigated in further studies in more detail. They may focus on hypothermic lung perfusion using antiinflammatoricy agents in the perfusat of the lungs.

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