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Role of poly(ADP-ribose) polymerase activation in the pathogenesis of cardiopulmonary dysfunction in a canine model of cardiopulmonary bypass

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Abstract

Objective: To investigate the effects of PARP inhibition on cardiac and pulmonary function during reperfusion in a clinically relevant experimental model of cardiopulmonary bypass. **Methods**: Twelve anesthetized dogs underwent hypothermic cardiopulmonary bypass. After 60 min of hypothermic cardiac arrest, reperfusion was started after application of either saline vehicle (control, $n = 6$), or the potent PARP-inhibitor PJ34 (5 mg/kg; $n = 6$). Biventricular hemodynamic variables were measured by combined pressure–volume–conductance catheters. Coronary and pulmonary blood flow, vasodilator responses to acetylcholine and sodium-nitroprusside and pulmonary function were also determined. The cardiac and pulmonary activation of PARP was detected by poly(ADP-ribose) immunohistochemistry. **Results**: Administration of PJ34 led to a significantly better recovery of left and right ventricular systolic function ($P < 0.05$) after 60 min of reperfusion. Coronary blood flow was also significantly higher in the PJ34 treated group ($P < 0.05$). PJ34 treatment preserved the acetylcholine-induced increases in coronary and pulmonary blood ($P < 0.05$). Pulmonary function in terms of alveolar arterial oxygen difference was better maintained in the PJ34 treated animals ($P < 0.05$). Immunohistochemical staining revealed PARP activation after cardiopulmonary bypass in both the heart and lung, which was prevented by PJ34. **Conclusions**: PARP inhibition improves the recovery of myocardial and endothelial function after hypothermic cardiac arrest and protects against the development of remote pulmonary injury during cardiopulmonary bypass.
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Keywords: Cardiopulmonary bypass; Reperfusion injury; PARP inhibition; Endothelial function

1. Introduction

The activation of poly(ADP-ribose) polymerase (PARP) is now considered a final common effector in various types of tissue injury including systemic inflammation, circulatory shock and ischemia/reperfusion [1]. Free radical and oxidant production and related cytotoxicity during ischemia/reperfusion leads to DNA strand-breakage which activates the nuclear enzyme PARP and initiates an energy consuming, inefficient cellular metabolic cycle with transfer of the ADP-ribosyl moiety of NAD^+ to protein acceptors. Both the genetic disruption of the PARP pathway and the pharmacological blockade of PARP effectively protect against oxygen and nitrogen-derived oxidant toxicity in cultured cells and attenuate regional myocardial ischemia/reperfusion and global hypoxia/reoxygenation injury [1–4]. It was also demonstrated that PARP inhibition leads to a significant improvement of endothelial function ex vivo in peroxynitrite-treated thoracic aortic rings and in isolated mesenteric arteries in the setting of splanchnic

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ischemia/reperfusion [1]. In our previous rat heart transplant study [5], we showed that hypothermic cardiac arrest and reperfusion lead to the activation of PARP and energy depletion. In this setting, the blockade of PARP with the novel potent PARP inhibitor PJ34 improved myocardial and endothelial functional recovery.

As routine cardiac surgery with cold cardioplegic arrest leads to a temporary cardiac dysfunction [6], we have now designed experiments to test the hypothesis that PARP-inhibition improves myocardial and endothelial recovery in a clinically relevant canine model of cardiopulmonary bypass (CPB) with hypothermic cardiac arrest. CPB is also known to induce a systemic inflammatory reaction [7] with free radical release [8] leading to secondary organ injury which may affect all vital organs including the brain, kidneys, lungs and gut. In addition to the systemic inflammatory reaction the lungs are not fully perfused because of reduction up to complete cessation of the pulmonary artery flow during CPB [9,10]. Accordingly, postoperative pulmonary dysfunction is a significant clinical problem, ranging from subclinical functional changes in most patients to full-blown adult respiratory distress syndrome in $<2\%$ of cases after CPB [9]. As PARP inhibition is a successful therapeutic concept in the treatment of a wide variety of inflammatory diseases, in the current study we have also focused on remote pulmonary injury. We hypothesized that PARP activation may also occur in the lungs after CPB and that PARP inhibition improves pulmonary function.

2. Materials and methods

2.1. Animals and experimental groups

Twelve dogs (foxhounds) weighing 25–35 kg (27 $\pm$ 4 kg) were used in this experiment. All animals received humane care in compliance with the European Convention on Animal Care. The experiments were approved by the ethical committee of the Land Baden-Württemberg for Animal Experimentation. Six animals received 5 mg/kg PJ34, a novel, potent, water-soluble phenanthridinone derivative PARP inhibitor [5,10,11] as a short infusion starting 5 min before aortic declamping and continued during the first 15 min of reperfusion. Six vehicle-treated animals served as controls. The protocol was carried out in blinded and randomized manner. The applied dose of PJ34 is based on our previous ischemia/reperfusion and pharmacokinetic studies [5].

2.2. General management and cardiopulmonary bypass

The dogs were premedicated with propionylpromazine and anesthetized with pentobarbital (15 mg/kg initial bolus and then 0.5 mg/kg per hour i.v.), paralyzed with pancuronium bromide (0.1 mg/kg as a bolus and then 0.2 mg/kg per hour i.v.) and endotracheally intubated. The dogs were ventilated with a mixture of room air and O_2 ($FiO_2 = 60\%$) at a frequency of 12–15 min^{-1} and a tidal volume starting at 15 ml/kg per minute. The settings were adjusted to maintain partial carbondioxide pressure levels between 35 and 40 mmHg except the time of the assessment of pulmonary gas exchange function. The femoral artery and vein and the external jugular vein were cannulated to record aortic pressure and central venous pressure as well as to take blood samples for biochemical analysis. Basic intravenous volume substitution was carried out with Ringer's solution (1 ml/min per kg). According to the values of potassium, bicarbonate and base excess, substitution included administration of potassium chloride and sodium bicarbonate (8.4%). Neither catecholamines nor other hormonal or pressor substances were administered.

After left anterolateral thoracotomy in the fourth intercostal space and pericardiotomy, the great vessels were dissected. After systemic anticoagulation with sodium heparin (300 U/kg) the left subclavian artery was cannulated for arterial perfusion. The venous cannula was placed in the right atrium. The extracorporeal circuit consisted of a heat exchanger, a venous reservoir, a roller pump and a membrane oxygenator primed with Ringer lactate solution (1000 ml) supplemented with heparin (150 U/kg) and 20 ml sodium bicarbonate (8.4%). After initiation of CPB, the body temperature was cooled to 28 °C. After crossclamping of the aorta, the heart was arrested with 25 ml/kg HTK solution (in mmol: 15 NaCl, 9 KCl, 4 $MgCl_2 \cdot 6H_2O$, 18 histidine hydrochloride monohydrate, 180 histidine, 2 tryptophan, 30 mannitol, 0.015 $CaCl_2$, 1 potassium-hydrogen-2-oxopentandioat, H_2O). During cardiac arrest the pump flow was set at 100 ml/kg per min to maintain perfusion pressure above a value of 35–40 mmHg at any time point and alpha-stat management was applied. Twenty minutes prior to crossclamp removal, rewarming was initiated. After 60 min of cardiac arrest, the aorta was declamped and the heart was reperfused with normothermic blood in the bypass circuit. If necessary, ventricular fibrillation was counteracted with DC cardioversion of 40 J. Ventilation was restarted with 60% oxygen and then adjusted to maintain arterial partial oxygen pressure levels above 60 mmHg and partial carbondioxide pressure levels between 35 and 40 mmHg except the time of the assessment of pulmonary gas exchange function. All animals were weaned from CPB without inotropic support 20 min after the release of the aortic crossclamp. Each animal underwent 90 min of CPB with 60 min of cardiac arrest.

2.3. Cardiac function

Left end right ventricular systolic and diastolic pressures and volumes were measured by combined 6F Millar pressure–conductance catheters with 6 mm spacing which were inserted via the apex and the pulmonary artery, respectively. Stroke volume (SV) was calculated from

the integrated flow signal measured by an aortic ultrasonic flow probe and was used to calibrate the volume signal from the conductance catheter. Parallel conductance was estimated by rapid injection of 1 ml of hypertonic saline into the pulmonary artery or superior vena cava, respectively. Vena cava occlusions were performed to obtain a series of pressure–volume loops. The slope (Ees) and intercept (V0) of the left and right ventricular end-systolic pressure–volume relationships and preload recruitable stroke work (PRSW) were calculated as load-independent indices of myocardial contractility.

Coronary blood flow was measured on the left anterior descendent artery with a perivascular ultrasonic flow probe. Coronary vascular resistance was calculated as the difference between mean aortic pressure and central venous pressure divided by coronary blood flow. Coronary endothelium-dependent vasodilatation was assessed after intracoronary administration of a single bolus of acetylcholine (ACH, 10^{-7} M) and endothelium-independent vasodilatation after sodium-nitroprusside (SNP, 10^{-4} M). The vasoresponse was expressed as percent change of baseline coronary vascular resistance.

2.4. Pulmonary function

Beside routine blood gas analysis blood gases were determined before and 1 h after weaning from CPB. Blood gas analysis was performed in steady state at room air ventilation and 40, 60, 80 and 100% oxygen ventilation at a fixed rate of 12 $\min^{-1}$. Pulmonary function was characterized by the alveolar-arterial oxygen difference which was calculated according to the standard formulas.

Pulmonary arterial pressure was measured by a 5F Millar catheter in the left pulmonary artery. In addition, the left lower lobar pulmonary artery was dissected and blood flow was measured by an 4 mm diameter ultrasonic flow probe, pulmonary vascular resistance was calculated as the difference between mean pulmonary arterial pressure and left atrial pressure. For the measurement of left atrial pressure as additional 5F Millar catheter was introduced into the left atrium. Pulmonary endothelium-dependent vasodilatation was assessed after intraarterial administration of a single bolus of ACH (10^{-7} M) and endothelium-independent vasodilatation after SNP (10^{-4} M). The vasoresponse was expressed as percent change of baseline pulmonary vascular resistance.

2.5. Biochemical assessment

Blood samples were taken from the femoral artery to measure creatinine phosphokinase (CPK) and the myocardial isoenzyme fraction (CPK-MB) as a marker of myocardial injury with standard photometry.

2.6. Poly(ADP-ribose) immunohistochemistry

Poly(ADP-ribose) (PAR) the product of PARP was detected in order to assess the activation of PARP [5]. Heart and lung biopsy specimens were taken at baseline and after 60 min reperfusion and fixed in formalin and embedded in paraffin. After section of the probes, slides were deparaffinized, antigen was retrieved by incubation in boiling 0.1 M sodium citrate (pH 6), then slides were rinsed in water. Slides were incubated in 10% (w/v) trichloro-acetic acid for 10 min to prevent catabolism of the polymer by poly(ADP-ribose) glycohydrolase. Slides were rinsed in PBS, then endogenous peroxidase activity was quenched with 1.5% (v/v) hydrogen peroxide in methanol for 15 min. Non-specific binding sites were blocked using 2% (v/v) normal goat serum in PBS for 1.5 h at 37 °C. Preliminary experiments determined optimal antibody concentrations. Chicken antibody against PAR was a generous gift from Dr John R. Simon (Tulip BioLabs, Inc. West Point, PA) and it was used 1:250 or 1:500 dilutions, slides were incubated overnight at 4 °C, then washed in PBS and as a secondary antibody, biotinylated goat anti-chicken IgG (Vector Laboratories, Burlingame, CA) was used for 30 min at 30 °C. After PBS washes, slides were incubated with VECTASTAIN Elite ABC (peroxidase) standard kit (Vector Lab.) for 30 min at 30 °C, and developed using diaminobenzidine substrate. Slides were counterstained with nuclear fast red.

2.7. Data analysis

All measurements were performed before CPB and after 60 min of reperfusion.

All values were expressed as mean ± standard error (SEM). Paired *t*-test was used to compare two means within groups. Individual means between the groups were compared by one-way analysis of variance followed by an unpaired *t*-test with Bonferroni correction for multiple comparisons and the post hoc Scheffe's test. A probability value less than 0.05 was considered statistically significant.

3. Results

Hemodynamic variables are shown in Table 1. Baseline parameters did not differ between the groups and were within the physiological range. Mean blood pressure during CPB was 58 ± 6 vs. 65 ± 7 mmHg in the control and in the PARP group, respectively (n.s.). After 60 min of cardioplegic arrest and 60 min of reperfusion, HR did not change in the control, or in the PJ34 treated group. Mean aortic pressure, left ventricular systolic pressure and SV decreased significantly in the control group while it remained unchanged in the PJ34 group. It should also be noted that cardiac showed a clear decreasing tendency within the control group without reaching the level of significance

Table 1
Hemodynamic variables before cardiopulmonary bypass and after 60 min of reperfusion

	Baseline		60 min of reperfusion	
	Control	PJ34	Control	PJ34
HR (beats/min)	110 ± 6	112 ± 7	127 ± 9	113 ± 8
MAP (mmHg)	96 ± 9	92 ± 9	70 ± 6*	86 ± 6**
LVSP (mmHg)	119 ± 8	114 ± 11	92 ± 7*	105 ± 7
LVEDP (mmHg)	8 ± 2	7 ± 2	8 ± 3	9 ± 2
CO (ml/min per kg)	96 ± 9	105 ± 8	85 ± 9	93 ± 10
SV (ml)	24 ± 2	22 ± 5	16 ± 2*	20 ± 4
CBF (ml/min)	32 ± 6	30 ± 5	21 ± 3*	34 ± 4**
PBF (ml/min)	452 ± 44	460 ± 60	413 ± 34	447 ± 52
PAP (mmHg)	12 ± 4	13 ± 5	12 ± 2	14 ± 3

HR, heart rate; MAP, mean aortic pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; CO, cardiac output; SV, stroke volume; CBF, coronary blood flow; PBF, pulmonary blood flow measured on the left lower lobar pulmonary artery; PAP, mean pulmonary arterial pressure. All values are given as mean ± SEM, $*P < 0.05$ vs. baseline, $**P < 0.05$ PJ34 vs. control.

(Table 1). Left and right ventricular Ees and PRSW fell significantly below the baseline values in the control group and were significantly lower than in the PJ34 group (Figs. 1 and 2). Heart rate, left ventricular end-diastolic pressure as well as pulmonary blood flow and resistance, parameters did not differ significantly between the groups.

Coronary blood flow was similar in both groups before cardioplegic arrest. After 60 min of reperfusion, coronary blood flow decreased significantly in the control group, while it remained unchanged in the PJ34 group (Table 1). Endothelium-dependent vasodilatation after ACH was significantly reduced in both groups after 60 min of reperfusion in comparison to pre-CPB values (Fig. 3). However, this decrease was significantly smaller in the PJ34 group. Endothelium-independent vasodilatation after SNP

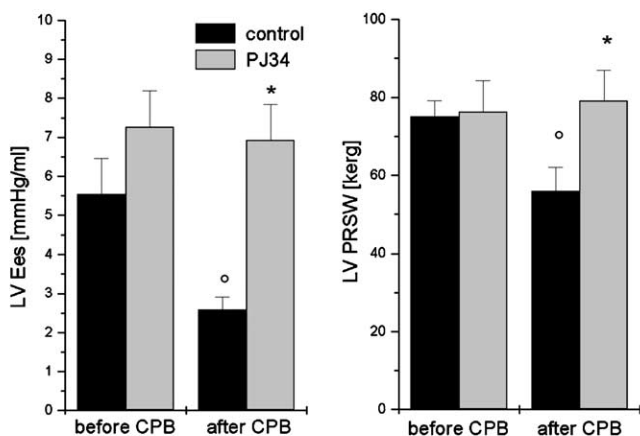

Fig. 1. The slope of the left ventricular (LV) end-systolic pressure–volume relationship (Ees, *left*) and the preload recruitable stroke work (PRSW, *right*) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SEM, $^{\circ}P < 0.05$ vs. baseline, $*P < 0.05$ PJ34 vs. control.

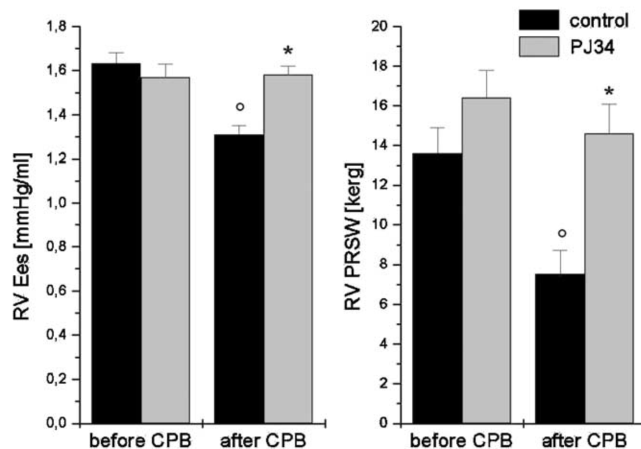

Fig. 2. The slope of the right ventricular (RV) end-systolic pressure–volume relationship (Ees, *left*) and the preload recruitable stroke work (PRSW, *right*) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SEM, $^{\circ}P < 0.05$ vs. baseline, $*P < 0.05$ PJ34 vs. control.

showed no significant differences over the time and between the groups (Fig. 3).

CPK and CPK-MB levels showed a significant increase in the control group after CPB, while the slight increase in the PJ34 group did not reach the level of significance (Fig. 4).

Fig. 5 shows alveolar arterial oxygen difference before and after CPB. Partial oxygen tension, oxygen saturation and alveolar arterial oxygen difference decreased significantly in the control group and they were significantly lower in comparison to the PJ34 group. Partial carbondioxide tension showed no significant difference between the groups.

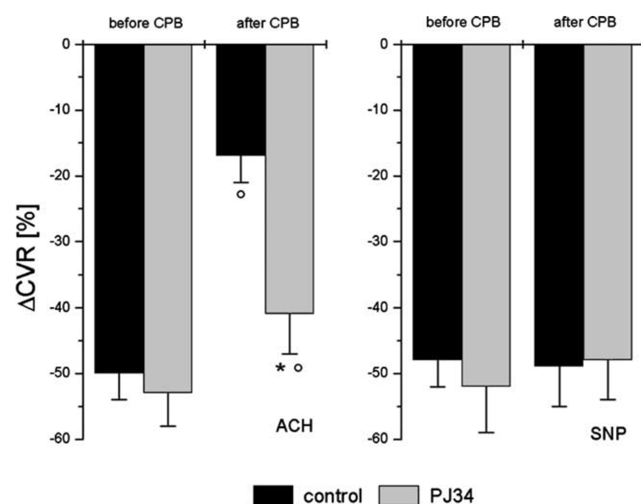

Fig. 3. Coronary vascular function. Endothelium dependent vasodilatation after acetylcholine (ACH, 10^{-7} M, *left*) and endothelium-independent vasodilatation after sodium-nitroprusside (SNP, 10^{-4}, *right*) expressed as percent change of coronary vascular resistance (CVR) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SEM, $^{\circ}P < 0.05$ vs. baseline, $*P < 0.05$ PJ34 vs. control.

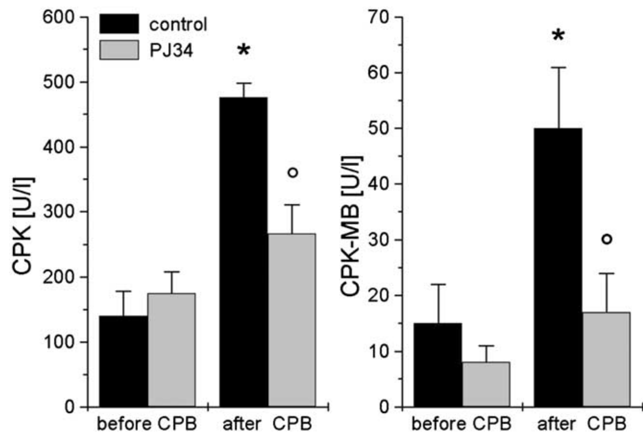

Fig. 4. Creatinine phosphokinase (CPK, right panel) and the myocardial isoenzyme fraction (CPK-MB, left panel) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SEM, $^{\circ}P < 0.05$ vs. baseline, $*P < 0.05$ PJ34 vs. control.

The values of PAP and PBF are shown in Table 1. PAP and PBF measured at the left lower lobar pulmonary artery were similar in both groups at baseline and remained stable after CPB. During aortic crossclamping no effective flow was detected in the pulmonary artery. Vascular responses to ACH and SNP did not differ between the groups at baseline. Pulmonary resistance showed a significantly attenuated response to injection of ACH after CPB in the control group while it remained unchanged in the PJ34 group (Fig. 6). Endothelium-independent vasodilatation after SNP did not differ between the groups and over the time.

There was no detectable PAR at baseline in the heart and lungs (Fig. 7). After 60 min of cardioplegic arrest and CPB, no PAR staining was found in the heart. In contrast, the lungs showed marked PAR staining. After 60 min of reperfusion extensive PAR staining was observed in both the heart and the lung in controls, and this staining was markedly abrogated in the PJ34 treated group.

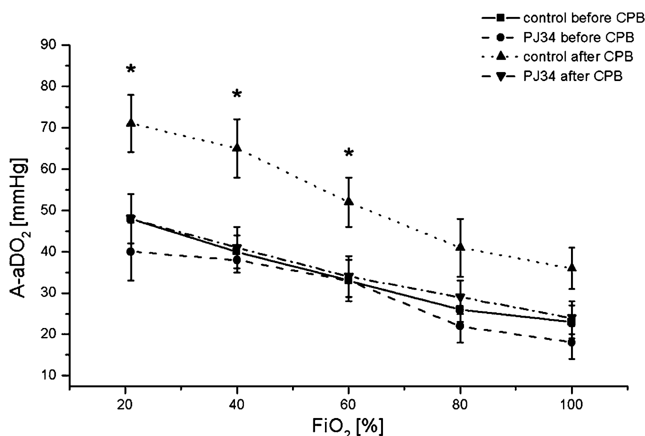

Fig. 5. Pulmonary gas exchange. Alveolar-arterial oxygen difference (A-aDO_2) at room air ventilation and 40, 60, 80 and 100% oxygen ventilation (FiO_2) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SEM, * indicates both $P < 0.05$ after vs. before CPB and $P < 0.05$ PJ34 vs. control.

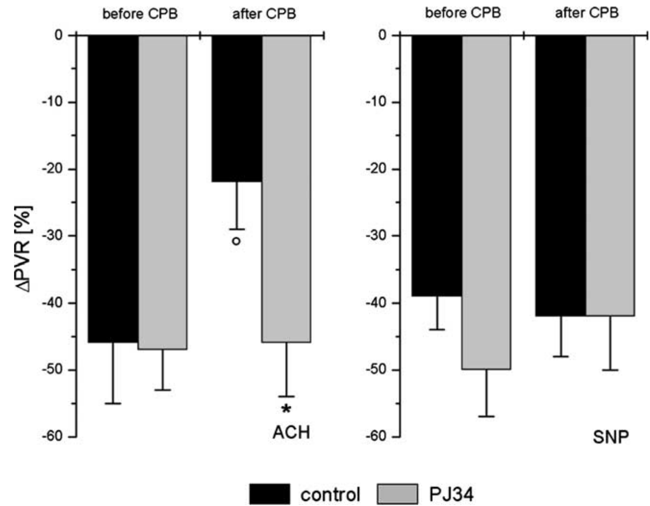

Fig. 6. Pulmonary vascular function. Endothelium dependent vasodilatation after acetylcholine (ACH, 10^{-7} M, *left*) and endothelium-independent vasodilatation after sodium-nitroprusside (SNP, 10^{-4}, *right*) expressed as percent change of pulmonary vascular resistance (PVR) before and after cardiopulmonary bypass (CPB) at 60 min of reperfusion. All values are given as mean ± SEM, $^{\circ}P < 0.05$ vs. baseline, $*P < 0.05$ PJ34 vs. control.

4. Discussion

The present study clearly demonstrate PARP activation in the reperfused heart after global hypothermic ischemia in an in vivo large animal model which was effectively prevented by the selective PARP inhibitor PJ34. It should be noted that PARP activation occurred not during ischemia but only during reperfusion. In the lungs, PARP activation could be shown during the time of cardiac arrest and aortic crossclamping which probably reflects to the systemic inflammatory reaction during CPB. PARP activation still persisted in the lungs during reperfusion, which was reversed by PJ34. We have shown here that PARP inhibition improves biventricular and endothelial functional recovery after cardioplegic arrest in a canine model of CPB. In addition, pulmonary function was significantly improved after treatment with the PARP inhibitor PJ34. The fact that contractile function and coronary blood flow were completely restored while coronary endothelial function was still impaired in the PJ34 group indicates that either other mechanisms than those leading to PARP activation are also involved in endothelial injury after hypothermic cardiac arrest, or the endothelium is more vulnerable to the ischemic stimulus [5,12].

Previous studies have shown PARP-activation in the reperfused heart [1–4]. PARP-knock out animals [3,13] or animals treated with pharmacological PARP inhibitors [2,5,14] were significantly protected against the myocardial injury. The current one is the first study which shows the effectiveness of PARP-inhibition in a clinically relevant large animal model. In accordance with the literature [6], CPB with cardioplegic arrest resulted in a moderate decrease in some of the global hemodynamic parameters

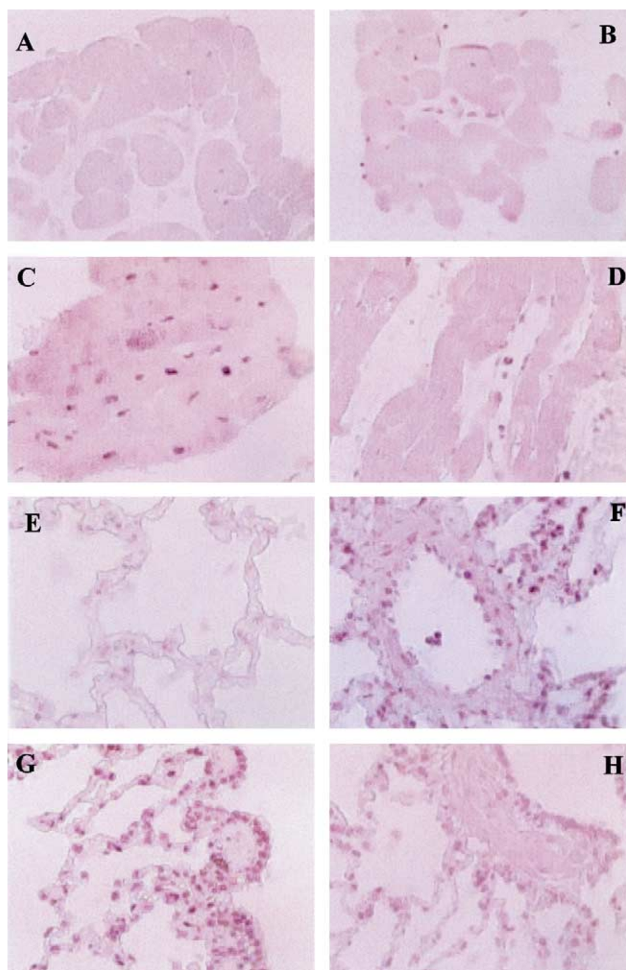

Fig. 7. Immunohistological staining against poly(ADP-ribose), a marker of poly(ADP-ribose) polymerase activation in the heart and lungs. Panel A shows representative poly(ADP-ribose) negative heart stainings before cardiopulmonary bypass (CPB). After 60 min of cardioplegic arrest (panel B), poly(ADP-ribose) staining was still negative in the heart. In panel C, strong positive poly(ADP-ribose) staining is present in the cell nuclei of control heart after CPB at 60 min of reperfusion which indicates poly(ADP-ribose) polymerase activation. Panel D shows heart representative heart specimen of the PJ34 treated group after CPB at 60 min of reperfusion. The poly(ADP-ribose) negative staining is indicative for the inhibition of poly(ADP-ribose) polymerase by PJ34 treatment. Panel E shows representative poly(ADP-ribose) negative lung stainings before CPB. After 60 min of cardioplegic arrest (panel F), poly(ADP-ribose) staining was positive in the cell nuclei of the lungs. In panel G, strong positive poly(ADP-ribose) staining is present in the control lungs after CPB at 60 min of reperfusion which indicates poly(ADP-ribose) polymerase activation. Panel H shows representative lung specimen of the PJ34 treated group after CPB at 60 min of reperfusion. The poly(ADP-ribose) negative staining is indicative for the inhibition of poly(ADP-ribose) polymerase by PJ34 treatment (400 × magnification).

in the control group. The approximately 30% decrease of SV was compensated by the increase of heart rate ensuring an unchanged cardiac output. Usually, decreased aortic or arterial pressure after CPB is caused by the combination of different factors: decreased cardiac function as a result of ischemia/reperfusion injury, peripheral vasodilatation in the context of the inflammatory response and very often intravascular hypovolemia [6]. As, however, filling pressures remained unchanged the increase of heart rate, the decrease of SV and mean aortic pressure in the control group may rather reflect to the changes of contractility and/or the peripheral vasodilatation. To differ between these factors, we assessed the slope of the end-systolic pressure volume relationships and PRSW as load-independent indices of myocardial contractility. These measurements clearly demonstrated a significant decrease of contractile function of both ventricles which is consistent with the literature [6]. In the present study, inhibition of PARP completely prevented contractile dysfunction as indicated by the assessed load-independent contractility indices. Secondary changes of global hemodynamics (i.e. changes of SV, heart rate and mean aortic pressure) as observed in the control group were abolished after application of PJ34.

The mechanisms of the PARP inhibitor's protective action in myocardial injury are multiple and have recently been overviewed [1,15]. PARP activation was identified as a key pathway in different pathophysiological conditions and disease states. PARP has a complex role in DNA damage-induced cell death. Much of the cell death related literature focuses on PARP cleavage (as opposed to PARP activation). PARP cleavage by caspases is a marker of apoptotic cell death, and has been shown to occur in various models of myocardial ischemia-reperfusion injury. The pathway described in the current article has no relationship to the PARP cleavage pathway: pharmacological inhibition of PARP inhibits the process of cell necrosis (rather than apoptosis). In fact, the cleaved form of PARP is catalytically inactive: PARP cleavage has been considered as an endogenous mechanism that serves to prevent PARP-dependent metabolic suppression and necrosis. Depending on the severity of DNA damage, genotoxic stimuli can trigger three different pathways. In the case of mild DNA damage, PARP facilitates DNA repair and thus survival. More severe DNA damage induces apoptotic cell death during which caspases, the main executor enzymes of apoptotic process, inactivate PARP cleaving into two fragments (p89 and p24). This pathway allows cells with irreparable DNA damage to become eliminated in a safe way. The most severe DNA damage may cause excessive PARP activation inhibiting glycolysis and mitochondrial respiration and depleting NAD^+ and ATP stores [1,2,5]. NAD^+/ATP depletion blocks apoptosis and results in necrosis. The mechanisms leading to tissue injury and organ dysfunction after ischemia/reperfusion or hypoxia/reoxygenation are multiple. However, there is good evidence that reactive oxygen species such as superoxide anions, hydroxyl radicals and hydrogen peroxide, as well as the reactive nitrogen species peroxynitrite contribute to reperfusion injury in the previously ischemic myocardium which in turn leads to PARP activation with subsequent myocardial and vascular injury [1,15]. In various types of ischemia/reperfusion, the prevention of PARP activation results in a better preservation of the high energy phosphate

content resulting in an improved energy status [1–5]. Beside its direct effects on myocardial metabolism, PARP-activation contributes to the expression of P-selectin and ICAM-1 during cardiac ischemia/reperfusion [2,5] and consequently to the recruitment of neutrophils into jeopardized tissue.

Though numerous in vitro and in vivo studies investigated the role of PARP activation in myocardial ischemia/-reperfusion injury only very few information exists about the effects on endothelial function. In endothelial cell cultures and isolated vessel rings, inhibition of PARP decreased or even prevented endothelial injury [1,5,10] and improved endothelial function [1,5] after different types of stimuli. In a recent study, the acute reversal of diabetic endothelial dysfunction was reported after a single treatment with the PARP inhibitor PJ34 [11]. We described for the first time in our previous study, that endothelial dysfunction is improved after PARP inhibition in heterotopically transplanted rat hearts subjected to ischemia/reperfusion. In accordance with these previous findings, we demonstrated a significant improvement of coronary endothelial function in an in vivo large animal model with direct coronary flow measurements. The mechanisms, leading to an improvement of endothelial function after PARP inhibition are discussed above and include both the improvement of the energetic status of the endothelial cells [1] end the reduction of neutrophil–endothelial interaction [2,5].

As pulmonary dysfunction is common after cardiac surgery [9], we assessed pulmonary function in terms of blood gas analysis and alveolar-arterial oxygen gradient, pulmonary blood flow as well as pulmonary endothelial function. In agreement with the literature [9,16–20], we observed impaired gas exchange and pulmonary endothelial dysfunction after extracorporal circulation in the control group. Massoudy et al. [18] showed in a clinical study that proinflammatory cytokines are increased in pulmonary venous blood and, at the same time, activated blood cells are retained in the pulmonary circulation. This indicates an inflammatory response of the lungs to extracorporal circulation. Schlensak et al. [19] clearly demonstrated that CPB caused a reduction in bronchial arterial blood flow which was associated with injury of the lungs. A recent histologic study described interstial edema, leakage of erythrocytes into the alveolar space and swelling of endothelial cells after CPB [20] in relation to free radical generation. Summarizing the recent literature, pulmonary dysfunction after CPB is the result of multiple insults which include general anesthesia, thoracotomy and breach of the pleura as well as blood contact with artificial material, hypothermia, pulmonary ischemia and lung ventilatory arrest [7,9]. Many of these factors may induce an inflammatory cascade with subsequent free radical production and PARP activation. The histological examinations clearly demonstrated that significant PARP activation occurs in the lungs during CPB, which can be reversed by the selective PARP inhibitor PJ34. There are only few studies, which describe the effects of PARP inhibition in lung injury. Cuzzocrea et al. [21] reported a reduction of lung injury and attenuated expression of P-selectin and ICAM-1 as well as the recruitment of neutrophils into injured lung after intrathoracic application of zymosan. Lipopolysaccharide induced lung injury was reduced by PJ34 in a recent study [22]. In rabbit pneumocytes, PARP inhibition preserves surfactant synthesis after hydrogen peroxide exposure [23]. The present study is the first one, which demonstrates a functional improvement by PARP-inhibition after lung injury in the setting of CPB.

An important issue is the different time pattern of PARP activation in the heart and the lungs during and after CPB. PARP activation was not present during the ischemic period in the heart, but during reperfusion, which indicates that the primary pathophysiologic processes leading to PARP activation are taking place during reperfusion. However, in completely different models of chronic regional ischemia and myocardial infarction, PARP activation may also occur in the ischemic zone [15]. In the lungs, PARP positivity was found after 60 min of CPB (at the end of aortic crossclamp time). During this time the lungs are not completely ischemic, but hypoperfused [19] and subjected to the systemic inflammatory reaction which results in early PARP activation. The present study showed for the first time that PARP activation in the context of CPB-induced lung injury can be reversed and pulmonary dysfunction can be prevented even after delayed application of a PARP inhibitor.

The present study has some limitations. Similarly to the clinical situation, in the present in vivo model, different types of pathologic stimuli are acting in concert: these include cardiac ischemia/reperfusion and systemic inflammatory response to CPB. Therefore, it remains unclear, to which extent the improved pulmonary function is a consequence of improved cardiac function in the PJ34 treated group, as opposed to being local effects of PARP-inhibition. Even if load-independent indices showed a marked difference between the groups, cardiac output, arterial pressure and pulmonary blood flow showed, if any, only small differences. Furthermore, immunohistochemistry demonstrated clear PARP activation in the lungs after CPB in the control group, which was prevented by PJ34. Therefore, we can assume that the observed improved pulmonary function is caused at least partly by the local effects of PARP inhibition.

In summary, the current results demonstrate that, in a clinically relevant large animal model of CPB, the potent PARP inhibitor PJ34 markedly attenuates reperfusion injury, resulting in a better functional cardiac recovery and improved pulmonary function. The pathogenetic mechanisms, namely inflammatory reaction and free radical generation, which lead to PARP activation, occur mainly during the reperfusion phase. PARP activation may last or even progress during the first 24–72 h after the initial

ischemic stimulus [1]. Due to its dual action against both ischemia/reperfusion and systemic inflammatory injury PARP inhibitors may be useful to reduce cardiac and other tissue injury specifically during cardiac surgery.

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