Poly(ADP-ribose) polymerase inhibitor PJ-34 reduces mesenteric vascular injury induced by experimental cardiopulmonary bypass with cardiac arrest

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Andrási, Terézia B., Anna Blázovics, Gábor Szabó, Christian F. Vahl, and Siegfried Hagl. Poly(ADP-ribose) polymerase inhibitor PJ-34 reduces mesenteric vascular injury induced by experimental cardiopulmonary bypass with cardiac arrest. Am J Physiol Heart Circ Physiol 288: H2972–H2978, 2005. First published January 28, 2005; doi:10.1152/ajpheart.01039.2004.—The aim of this study was to investigate effects of poly(ADP-ribose) polymerase (PARP) inhibition on mesenteric vascular function and metabolism in an experimental model of cardiopulmonary bypass (CPB) with cardiac arrest. Twelve anesthetized dogs underwent 90-min hypothermic CPB. After 60 min of cardiac arrest, reperfusion was started for 40 min following application of either saline vehicle (control, n = 6) or a potent PARP inhibitor, PJ-34 (10 mg/kg iv bolus and 0.5 mg·kg−1·min−1 infusion for 20 min, n = 6). PJ-34 led to better recovery of cardiac output (2.2 ± 0.1 vs. 1.8 ± 0.2 l/min in control) and mesenteric blood flow (175 ± 38 vs. 83 ± 4 ml/min, P < 0.05 vs. control) after reperfusion. The impaired vasodilator response of the superior mesenteric artery to acetylcholine, assessed in the control group after CPB (−32.8 ± 3 vs. −57.6 ± 6.6% at baseline, P < 0.05), was improved by PJ-34 (−50.3 ± 3.6 vs. −54.3 ± 4.1% at baseline, P < 0.05 vs. control). Although plasma nitrate/nitrite concentrations were not significantly different between groups, mesenteric nitric oxide synthase activity was increased in the PJ-34 group (P < 0.05). Moreover, the treated group showed a marked attenuation of mesenteric venous plasma myeloperoxidase levels after CPB compared with the control group (75 ± 1 vs. 135 ± 9 ng/ml, P < 0.05). Pharmacological PARP inhibition protects against development of post-CPB mesenteric vascular dysfunction by improving hemodynamics, restoring nitric oxide production, and reducing neutrophil adhesion.

endothelial function; nitric oxide; neutrophil adhesion; hemodynamics

There is a large body of evidence suggesting that the mesenteric vasculature has to be considered a primary target in the development of gastrointestinal complications after surgical procedures involving cardiopulmonary bypass (CPB) and cardiac arrest (1, 8, 31, 32). Indeed, CPB is known to induce a systemic inflammatory reaction with free radical release leading to endothelial dysfunction in the mesenteric micro- and macrocirculation (1, 31). Factors that are related to this damage include reduced expression and activity of the endothelial nitric oxide synthase (eNOS) and reduced L-arginine availability that results in reduced bioavailability of NO (2, 15). Moreover, increased production of superoxide leads to increased degradation of NO and the formation of oxidant peroxynitrite, a potent trigger of various forms of oxidative cell injury (4, 20).

Current evidence suggests that an important candidate pathway of free radical-induced endothelial vascular injury involves the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 inhibitors have been shown to diminish brain damage after cerebral ischemia (30), prevent myocardial injury in heart ischemia (28), and reduce renal ischemia-reperfusion injury (18). In addition, several recent reports indicate that PARP inhibition counteracts the mesenteric dysfunction in ischemia-reperfusion (17), colon inflammation (35), and hemorrhagic (16) and endotoxemic shock (25).

Because most of the postoperative medication presently used to maintain and/or improve left ventricular function after cardiac resuscitation exerts a deleterious effect on the mesenteric circulation (4, 33), the question arises whether the novel, potent cardioprotective (28, 29), water-soluble phenanthrinone derivative PJ-34 [N-(6-oxo-5,6-dihydrophenanthridin-2-yl)-N,N-dimethylacetamide] may improve the mesenteric vascular function after CPB with cardiac arrest.

Materials and Methods

General Preparation

The experiments were approved by the Ethical Committee of the Land Baden-Württemberg for Animal Experimentation. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996).

After an overnight fast, 12 foxhound dogs were premedicated with propionylpromazine (4 mg/kg sc), and anesthesia was induced with pentobarbital sodium (15 mg/kg iv). After endotracheal intubation, anesthesia was maintained with continuous intravenous infusion of pentobarbital sodium (30 mg·kg−1·h−1), pirotiamide (0.2 mg·kg−1·h−1), and pancuronium (0.2 mg·kg−1·h−1). The dogs were ventilated with 60–100% oxygen-enriched room air at a respiratory rate between 10 and 18 breaths/min to maintain arterial oxygen tension >100 mmHg, arterial PCO2 between 35 and 40 mmHg, and pH in the range of 7.35 to 7.45. Sodium bicarbonate was administered intravenously to treat acidemia. Core body temperature was measured with a rectal probe and maintained at 36°C by using a heating blanket. A catheter was placed in the right femoral artery for pressure monitoring and blood sampling. The femoral vein was cannulated for fluid and drug administration.

Through a midline laparotomy, a 12-gauge ultrasonic flow probe (Transonic Systems, Ithaca, NY) was positioned around the origin of the superior mesenteric artery and connected to a flow monitor. A 2.5-F catheter was introduced in the superior mesenteric vein through an ileal tributary. The cannula was flushed with heparinized saline

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solution and secured with a purse-string suture. After preparation, the intestine was reintroduced into the abdominal cavity.

A lateral thoracotomy was performed in the fifth intercostal space, and a pericardial cradle was created to suspend the heart. After systemic anticoagulation with heparin sodium (300 U/kg), the left subclavian artery was cannulated for arterial perfusion. The venous cannula was placed in the right atrium. A small cannula for cardioplegic delivery was inserted into the root of the ascending aorta through a purse-string suture. An ultrasonic flow probe (Transonic Systems) was placed around the ascending aorta for monitoring of cardiac output. A catheter was placed into the left atrium for the administration of vasoactive drugs.

**CPB Management**

The extracorporeal circuit consisted of a heat exchanger (Normothermic; Stöckert Instrumente, Munich, Germany), a venous reservoir (Dideco D 764, 40-μm Midicard), a roller pump (type 10-40-00; Stöckert Instrumente), and a membrane oxygenator (Dideco D 701, Masterflo 34) primed with Ringer lactate solution (1,000 ml) supplemented with heparin (150 U/kg) and 20 ml of sodium bicarbonate (8.4%). After initiation of CPB, the body temperature was decreased to 28°C. The period of cooling lasted ~10 min.

Thereafter, the aorta was cross-clamped between the aortic perfusion cannula and the cardioplegia infusion cannula. The heart was vented, and cold crystalloid cardioplegic solution (1,000 ml) was delivered for 7 min. The composition of the cardioplegic solution was (in mmol) 15 NaCl, 9 KCl, 4 MgCl2·6H2O, 18 histidine hydrochloride monohydrate, 180 histidine, 2 tryptophan, 30 mannitol, 0.015 CaCl2·H2O, and 1 2-oxopentanoic calcium salt. During cardiac arrest, the pump rate was set at 100 ml·kg⁻¹·min⁻¹ to maintain the perfusion pressure at a value >40 mmHg. Arterial blood gas and pH measurements were performed before the initiation of cardiopulmonary bypass and at each 20 min thereafter. Alpha Stat methodology was applied. Substitution included administration of potassium chloride and sodium bicarbonate.

Twenty minutes before the anticipated cross-clamp 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. At a temperature of 33°C, ventricular fibrillation was counteracted with direct current cardioversion of 40 J. Ventilation was restarted with 100% oxygen. All animals were weaned from CPB without inotropic support 20 min after the release of the aortic cross clamp. Each animal underwent a 90-min CPB with 60-min cardiac arrest. After the cardiopulmonary bypass was discontinued, the hearts were allowed to beat fully for 30 min.

**Experimental Protocol**

Heart rate (HR), mean arterial blood pressure (MAP), cardiac output (CO), pump rate, and mesenteric blood flow (MBF) were monitored continuously throughout the experiments and recorded every 20 min. The vascular reactivity of the superior mesenteric artery to vasoactive drugs was determined. After each drug administration, recovery was permitted until the hemodynamic parameters returned to steady-state values. After completion of measurements, cardiopulmonary bypass was initiated.

At the time of the aortic declamping, the PJ-34 group (n = 6) received an intra-atrial bolus of 10 mg/kg PJ-34, followed by intravenous continuous infusion of 0.5 mg·kg⁻¹·min⁻¹ PJ-34 for 20 min. In the control group, saline solution was given in similar volumes.

Mesenteric venous and systemic arterial blood samples were simultaneously collected before cardiopulmonary bypass at 20, 40, and 60 min of cardiac arrest and at 20 and 40 min of reperfusion. Reperfusion consisted of two phases, 20 min of vented bypass followed by 20 min of intact circulation off pump. After 90 min, animals were set off from extracorporeal circulation. Thirty minutes later, determinations of mesenteric vascular responses to exogenous stimuli were repeated. Mesenteric arteriolar vessels of 2–3 mm in diameter were harvested before and after 90-min CPB.

**Measurements**

**Hemodynamics.** Mesenteric vascular resistance (MVR) was calculated as the rapport between MAP and MBF. CO values during cardiac arrest were considered identical to pump rate.

**Mesenteric vascular function.** Endothelium-dependent vascular reactivity of the superior mesenteric artery was determined by measuring vasodilator responses to single bolus doses of acetylcholine (ACh; 1 μmol). Endothelium-independent relaxation was studied using the NO-donor sodium nitroprusside (SNP; 0.01 mol). Drugs were injected into the left atrium. The peak vasodilator response occurred ~2 s after the rapid injection of the bolus. Vasorelaxation was expressed as relative variation of MVR compared with steady-state values.

**Gut function measurements.** Hemoglobin (Hb), PO2, PCO2, SO2, H2CO3, and pH were measured in the arterial and venous blood with the use of a blood-gas analyzer (AVL 995-Hb). The oxygen content of the systemic arterial blood (Cao2) and mesenteric venous blood (CsmvO2) was calculated using the standard equation [Hb (g/dl) × 1.39 × SO2] + (PO2 / 0.00314). Gut oxygen delivery (DO2) was determined as follows: 0.01 × MBF × Cao2. Gut oxygen consumption (VO2) was determined as follows: 0.01 × MBF × arteriovenous O2 content difference (Cao2 – CsmvO2). Mesenteric oxygen extraction was calculated as VO2/DO2 × 100.

**Nitric metabolites.** Plasma nitrate and nitrite levels were determined by enzyme-linked immunosorbent assays (ELISA) according to the Griess method by using reagents provided from IBL-Hamburg (Hamburg, Germany). Nitrate plus nitrite levels were determined after enzymatic conversion of nitrite to nitrate using nitrate reductase. Optical density at 540 nm (OD540) was measured (Labsystems iEMS Reader MF), and total nitrate plus nitrite and nitrate concentrations were calculated by comparison of the OD540 of the standard solutions of nitrate plus nitrite and of nitrite. Nitrate concentrations were calculated as the difference between nitrate plus nitrite and nitrate concentrations.

**NOS activity.** This assay was performed with the conversion of [3H]arginine to [3H]citrulline as a measure of NOS activity. Briefly, mesenteric arteriolar rings were homogenized in NOS assay buffer (50 mmol/l Tris·HCl, pH 7.5, containing 0.1 mmol/l EDTA and 0.1 mmol/l EGTA) with a protease inhibitor cocktail. Enzyme reactions were carried out at 37°C in the presence of total protein extracts (500 μg), 14 μmol/l tetrahydrobiopterin, 100 μmol/l FAD, 1 mmol/l MgCl2, 5 μmol/l unlabeled l-arginine, 15 mmol/l [3H]arginine, 25 units of calmodulin, and 5 mmol/l calcium. Assays were incubated for 60 min at 37°C. The reactions were stopped by the addition of iced stop buffer and then applied to columns containing 1 ml of Dowex AG50W-X8 resin (Na⁺ form, preequilibrated with 1 N NaOH). [3H]citrulline was then quantified with scintillation counting.

**Neutrophil adhesion.** The granules of neutrophils (~70% of the white blood cells) contain a large number of different enzymes. Myeloperoxidase (MPO) catalyzes the oxidation of substances through H2O2 and is a marker of inflammatory activity and oxidative stress in the gastrointestinal tract. We determined plasma MPO levels using ELISA. Peroxidase-labeled antibody against MPO was used (IBL-Hamburg). Absorption was determined with a plate reader at 450 nm. A dose-response curve of the absorbance unit (OD450) versus concentration was generated using results obtained from the calibration. MPO values from the measured samples were determined directly from this curve.

**Statistical Analyses**

Data were analyzed using the SAS System for Windows. Time-related differences were analyzed using two-way ANOVA for repeated measures. Single-event nonrepeated variables were compared between the groups by using the Wilcoxon test. Probability values were compared using the Kruskal-Wallis test.
\textit{RESULTS}

\textbf{Hemodynamic}

Hemodynamic measurements are summarized in Table 1. At baseline as well as after 20 (vented bypass) and 40 min (intact circulation off pump) of reperfusion, HR showed no statistical differences between groups. Peripheral perfusion was maintained in a physiological range during cardiac arrest by a pump rate closely similar to preoperative CO values (Table 1). After weaning from CPB, CO decreased significantly in the control group \((1.8 \pm 0.2 \text{ l/min}, P < 0.05)\) compared with baseline values \((2.4 \pm 0.4 \text{ l/min})\). Treatment with PJ-34 significantly improved CO \((2.2 \pm 0.1 \text{ l/min})\) after CPB \((P < 0.05 \text{ vs. control})\).

MAP decreased similarly in the control and treated groups after the initiation of CPB. At the time of reperfusion, a similar transient recovery of MAP \((P < 0.05)\) was assessed in both groups.

MBF was comparable between groups at baseline and was maintained at similar levels during cardiac arrest with no statistical difference between groups. Reperfusion was followed by a significant decrease in MBF in the control group \((83 \pm 4 \text{ vs. } 146 \pm 5 \text{ ml/min}, P < 0.05)\). Administration of PJ-34 significantly augmented MBF at 20 \((181 \pm 20 \text{ ml/min})\) and 40 min \((175 \pm 38 \text{ ml/min})\) of reperfusion compared with the control group \((P < 0.05)\).

MVR was not significantly influenced by the initiation of CPB; however, a slight decrease appeared in both groups during cardiac arrest (Table 1). After aortic declamping was completed, MVR tended to increase in the control group.

\textbf{Mesenteric Vascular Function}

Before CPB, single bolus administration of \(\text{ACH}\) produced similar vasorelaxations in both groups. Post-CPB vasorelaxation was significantly decreased in the control group \((-32.8 \pm 3.3 \text{ vs. } -57.6 \pm 6.6\% \text{ at baseline}, P < 0.05)\). As shown in Fig. 1A, administration of PJ-34 significantly improved the response to \(\text{ACH}\) \((-50.3 \pm 3.6\%, P < 0.05 \text{ vs. control})\), but without a complete restoration of the vasodilator effect \((-54.3 \pm 4.1\% \text{ at baseline})\). The endothelium-independent vascular function was neither significantly impaired after the 90-min CPB nor influenced by administration of PJ-34 (Fig. 1B).

\textbf{Gut Function Metabolism}

Oxygen metabolism was slightly decreased in the mesentery during cardiac arrest (Table 1). Administration of PJ-34 increased the oxygen delivery \((P < 0.05 \text{ vs. baseline})\) without influencing oxygen consumed after reperfusion. However, no differences in oxygen extraction were assessed after the CPB in the PJ-34 group compared with controls.

\textbf{NO Metabolism}

To identify NO production in the mesentery, we determined mesenteric NOS activity (Fig. 2). In the control animals, NOS activity after CPB \((164.4 \pm 12 \text{ mmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1})\) was comparable to pre-CPB values \((139.2 \pm 19 \text{ mmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1})\). A significant increase of NOS activity during the postresuscitation period \((P < 0.01)\) compared with baseline and the control group.

\textbf{Systemic Administration of PJ-34 Significantly Decreased MVR during the Postresuscitation Period (P < 0.01) Compared with Baseline and the Control Group.}

\subsection*{Table 1. Hemodynamic variables}

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>20 min CA</th>
<th>40 min CA</th>
<th>60 min CA</th>
<th>20 min RP</th>
<th>40 min RP</th>
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<tr>
<td>HR, beats/min</td>
<td>Control</td>
<td>130±3</td>
<td>138±14</td>
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<td></td>
<td>PJ-34</td>
<td>126±3</td>
<td>141±4</td>
<td>138±1</td>
<td>132±11</td>
<td>137±23</td>
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<td>CO, l/min</td>
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<td></td>
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<td>2.6±0.1</td>
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<td>2.3±0.1</td>
<td>2.3±0.1</td>
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<td>MAP, mmHg</td>
<td>Control</td>
<td>115±3</td>
<td>75±2*</td>
<td>60±2*</td>
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<td>76±2</td>
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<td></td>
<td>PJ-34</td>
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<td>61±5*</td>
<td>66±4*</td>
<td>73±5*</td>
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<td>MBF, ml/min</td>
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<td>146±5</td>
<td>122±3</td>
<td>115±2</td>
<td>116±4</td>
<td>111±4</td>
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<td></td>
<td>PJ-34</td>
<td>163±16</td>
<td>110±8</td>
<td>105±11</td>
<td>113±13</td>
<td>181±20*</td>
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<td>MVR, mmHg/ml·min·ml⁻¹</td>
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<td>0.79±0.02</td>
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<td>Pao₂, mmHg</td>
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<td>68±5</td>
<td>267±31*</td>
<td>247±14*</td>
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<td>181±21*</td>
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<td>PJ-34</td>
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<td>252±30*</td>
<td>234±15*</td>
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<td>128±36</td>
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<td>PVo₂, mmHg</td>
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<td>54.5±8</td>
<td>55.4±7.3</td>
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<td>PvcO₂, mmHg</td>
<td>Control</td>
<td>29.1±2.6</td>
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<td>59.4±5.4</td>
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<td>47.7±49</td>
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<td>DO₂, mmol/l</td>
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<td>14.2±2.1</td>
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<td>VO₂, mmol/l</td>
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<td>Ext O₂ %</td>
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<td>19.1±3.5</td>
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<td>23.9±5.3</td>
<td>28.9±7.8*</td>
<td>34.9±7.3*</td>
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</table>

Values are means ± SE. Parameters were measured at baseline and during cardiac arrest (CA) and reperfusion (RP). HR, heart rate; CO, cardiac output; MAP, mean arterial pressure; MBF, mesenteric blood flow; MVR, mesenteric vascular resistance; Pao₂, partial arterial oxygen pressure; PBV, partial venous oxygen pressure; Pao₂, partial arterial carbon dioxide pressure; PBV, partial venous carbon dioxide pressure; DO₂, mesenteric oxygen delivery; VO₂, mesenteric oxygen consumption; Ext O₂, mesenteric oxygen extraction. CO values during CA were considered identical to pump rate.*P < 0.05 vs. baseline. †P < 0.05 vs. control.
(\(P < 0.01\) vs. baseline) was assessed in the PJ-34 group after reperfusion (294.0 \(\pm\) 24 mmol·mg\(^{-1}\)·min\(^{-1}\)) compared with controls (\(P < 0.01\)) and baseline values (110.4 \(\pm\) 12 mmol·mg\(^{-1}\)·min\(^{-1}\), \(P < 0.01\)).

To assess the involvement of NO in the vascular function, we determined its nitrate and nitrite metabolites (Fig. 3). The mesenteric venous nitrate or nitrite levels remained constant in the control group during the experiments. Surprisingly, administration of PJ-34 did not influence the nitrate and nitrite production. However, the lower standard error of the mean for nitrite values demonstrates a higher specificity of this parameter compared with nitrate.

**Neutrophil Adhesion**

Assessment of neutrophil accumulation in the mesenteric circulation was performed by measurement of the activity of MPO, an enzyme specific to granulocyte lysosomes, and therefore directly correlated to the number of neutrophils (Fig. 4). Arterial plasma MPO level was significantly elevated during the reperfusion period (156.9 \(\pm\) 19.1 and 126.7 \(\pm\) 1.3 vs. 62 \(\pm\) 6 ng/ml at baseline, \(P < 0.01\)) in the control group, indicating an enhanced neutrophil adhesion stimulated by CPB. Administration of PJ-34 maintained the MPO level at baseline values during the reperfusion period (63.7 \(\pm\) 6.6 and 75.3 \(\pm\) 0.7 vs. 58.4 \(\pm\) 3.8 ng/ml at baseline, \(P < 0.01\) vs. controls).

**DISCUSSION**

In the present study we have shown that application of the novel PARP inhibitor PJ-34 during early reperfusion improves the post-CPB endothelial function in the mesenteric vascular circulation.

CPB per se causes a marked impairment of the endothelium-dependent relaxations of the superior mesenteric artery (Fig. 1A). In accordance with previous studies in our laboratory (1, 2), this effect is related to a reduced ability of the vascular endothelium to generate NO, not to a reduced ability of the vascular smooth muscle to relax in response to NO, because the relaxant effect of the NO donor compound SNP remained unaltered (Fig. 1B).

There is accumulating evidence demonstrating that pharmacological inhibition or genetic inactivation of PARP maintains endothelial integrity in the mesenteric vasculature under conditions of oxidative stress (7, 17, 25, 35). Triggered by peroxynitrite-induced DNA single-strand breaks, PARP catalyzes an energy-consuming polymerization of ADP-ribose, resulting...
in NAD depletion, inhibition of glycolysis and mitochondrial respiration, and the ultimate reduction of intracellular high-energy phosphates in endothelial cells, contributing to endothelial injury in isolated mesenteric arteries (19, 25). Therefore, improved endothelial function in our treated group after CPB can be explained, at least in part, by the improved energetic balance of the endothelium.

However, the mechanisms leading to vascular dysfunction after CPB are multiple. Because hemodynamic instability is common after CPB, we assessed some hemodynamic parameters during the experiments. In accordance with the literature (1, 2, 31), CPB with cardioplegic arrest resulted in a moderate decrease of MBF in our control group, caused by a combination of decreased CO and aortic arterial pressure as a result of cardiac dysfunction after ischemia-reperfusion injury.

However, the significant postreperfusion mesenteric vasomotor constriction (Table 1) was not observed in our treated animals. Moreover, the post-CPB decrease of CO was also prevented by PJ-34 administration. In light of these findings, the present data emphasize that by enhancing cardiac inotropism (29) and output, PJ-34 subsequently restores the blood supply to the mesenteric bed, reducing the malperfusion-associated ischemic injury.

Consistent with the beneficial hemodynamic effects, administration of PJ-34 also increased the oxygen delivery to the mesentery, but without influencing the mesenteric oxygen consume. Therefore, by restoring the physiological hemodynamic status, PJ-34 reduces not only the ischemic but also the reperfusion injury to the mesentery.

Besides the improvement of energetic balance and hemodynamic status, PARP inhibition in the early reperfusion phase may have additional effects that influence the long-term course of endothelial function after CPB. It has been previously shown that during the early phase of reperfusion after hypothermic CPB, endogenous production of NO is impaired (31), dependent on changes in eNOS and inducible NOS (iNOS) activity or gene expression (11, 21). In the current study we have found that in the mesenteric vasculature from control animals, the activity of NOS remains unchanged after CPB (Fig. 2). The rapid oxidation to nitrite, which quickly converts to nitrate (Fig. 3), is not increased. Recently, Hayashi et al. (12) demonstrated that a systemic increase of nitrates and nitrites was observed neither during nor immediately after the end of CPB but appeared 3 h after the end of CPB. Therefore, it is conceivable that mesenteric endothelial dysfunction in the early post-CPB period is caused by other mechanisms that may include decreased NOS substrate or increased turnover of NO into oxygen free radicals (11).

Conversely, the increased NOS activity in the treated group clearly demonstrates a significant enhancement in NO production. In a recent study (2), we found that exogenous l-arginine supplementation restores the mesenteric endothelial function after CPB, probably by increasing its intracellular availability. Therefore, we cannot exclude the possibility that PARP inhibition triggers the intracellular l-arginine uptake by a yet unknown mechanism, thus accelerating the NO production rate. In contrast, PARP activity has been shown to be involved in the process of both iNOS and eNOS expression in various experimental models (5, 23, 27). Although the specific mechanisms whereby PARP regulates the expression of iNOS and eNOS remain to be clarified, recent studies have proposed a role for PARP in the process of transcription (24, 26). In this regard, Khandoga et al. (14) demonstrated that intrahepatic iNOS expression is not changed during hepatic ischemia-reperfusion and is not affected by PARP inhibition or PARP gene deficiency. Tofukuji et al. (31) found unchanged iNOS expression in the ileum of dogs undergoing CPB. Later, Szabó et al. (24, 26, 27) demonstrated that PARP inhibition reduces iNOS expression in endothelial cells exposed to several injurious factors. In addition, Soriano et al. (22, 23) found that, surprisingly, eNOS expression is completely restored, and even more, the enzyme tended to be overexpressed by PARP inhibition in endothelial vascular cells from diabetic animals. Therefore, the increased total NOS activity in our experiments supports the idea that changes in iNOS and eNOS expression may be additional mechanisms whereby inhibition of PARP restores the physiological NO production. Because we were unable to detect an increase of the nitrate/nitrite production parallel to the increased NOS activity, one can speculate that PJ-34, by reducing oxidative stress, subsequently decreased the premature oxidation of NO and thus might indirectly prolong the lifetime of NO and its vasodilator effects. In addition, NO might be primarily stored in the form of other circulating intermediates such as nitrosothiols (13). Moreover, in the presence of reduced gastrointestinal mucosal pH after CPB, nitrate and nitrites also may release NO, which when achieving high concentrations may turn into free radicals.

However, the concern that increased NO production will result in the generation of free radicals, implicated in the pathogenesis of intestinal inflammation, was recently eliminated by Binion et al. (3), who demonstrated that even when high-output NO is produced by iNOS expression in the intestinal microvasculature, it can act to reduce oxidative stress (1). In contrast, the possibility cannot be excluded that PJ-34 may increase NO production from eNOS without providing adverse effects of iNOS activation. Therefore, inactivation of PARP may represent a novel strategy to counteract the energy failure and vascular dysfunction in pathophysiological conditions associated with eNOS/iNOS dysfunction.
Importantly, the endothelium-derived NO is a key inhibitor of neutrophil activation, adhesion, and transmigration. There also is accumulating evidence demonstrating that the regulation of endothelium-dependent relaxant ability by PARP is directly related to modulation of intracellular NADPH levels (10), with NADPH being an essential cofactor for NOS. Through the above mechanism, one can hypothesize that free radicals and oxidants injure the vascular endothelium, which reduces NO production, which then leads to neutrophil infiltration. Furthermore, leukocyte activation fuels the release of large amounts of oxygen free radicals during and after CPB (2, 9), which ultimately impair the availability of NO and alter the NO-receptor interactions, thus contributing to the mesenteric endothelial injury in our control group (positive feedback cycle). PARP inhibition, by interrupting this cycle, may reduce both neutrophil infiltration and free radical generation.

In support of this hypothesis, previous reports have shown that inhibition of PARP can interrupt the interaction between neutrophils and endothelial cells in the mesentery (19, 21), heart (34), and lung (6) by reducing the expression of P-selectin and the upregulation of ICAM-1. Moreover, Szabó and colleagues (22, 25) demonstrated that in the mesenteric microcirculation, inhibition of PARP modulates a postadhesion phenomenon, increasing the rate of adherent neutrophil detachment from the endothelium. However, it is unlikely that PARP directly regulates neutrophil function, because neutrophil granulocytes do not contain the PARP enzyme. Our results emphasize that P34 ameliorates neutrophil infiltration mainly by increasing NO production. An eventual increase of circulating nitrosothiols (13) also could strongly reduce platelet activation.

Accordingly, we present data in the current study demonstrating that inhibition of PARP with P34 reduces leukocyte accumulation in the mesenteric circulation after CPB (Fig. 4), indicating that this phenomenon is at least partly responsible for the early endothelial dysfunction in the mesentery. In summary, the salutary effect of pharmacological inhibition of PARP on mesenteric vascular function would therefore indicate that PARP activation takes place in the mesentery during CPB, thus being a highly sensitive, relatively early effector mechanism of the postoperative, nonocclusive ischemia-reperfusion injury. P34 reduces the post-CPB mesenteric vascular dysfunction via several pathways: 1) it restores the physiological blood supply to the mesentery after CPB; 2) it seems to increase endothelial NO production without altering its physiological metabolism; and 3) it blocks neutrophil adhesion, thus affecting multiple aspects of the inflammatory response to CPB. Therefore, pharmacological PARP inhibition with P34 during reperfusion represents a viable modality to achieve not only better heart function (28, 29) but also intestinal protection against the CPB-associated malperfusion damage. Further studies are warranted to test this beneficial effect in other models prone to develop postoperative, nonocclusive mesenteric ischemia due to global circulatory instability.

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