Poly(ADP-Ribose) Polymerase Inhibition Reduces Reperfusion Injury After Heart Transplantation


Abstract—The aim of the present study was to investigate the effects of the novel poly(ADP-ribose) polymerase (PARP) inhibitor PJ34 (N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide) on myocardial and endothelial function after hypothermic ischemia and reperfusion in a heterotopic rat heart transplantation model. After a 1-hour ischemic preservation, reperfusion was started either after application of placebo or PJ34 (3 mg/kg). The assessment of left ventricular pressure–volume relations, total coronary blood flow, endothelial function, myocardial high energy phosphates, and histological analysis were performed at 1 and 24 hours of reperfusion. After 1 hour, myocardial contractility and relaxation, coronary blood flow, and endothelial function were significantly improved and myocardial high energy phosphate content was preserved in the PJ34-treated animals. Improved transplant function was also seen with treatment with another, structurally different PARP inhibitor, 5-aminoisoquinoline. The PARP inhibitors did not affect baseline cardiac function. Immunohistological staining confirmed that PJ34 prevented the activation of PARP in the transplanted hearts. The activation of P-selectin and ICAM-1 was significantly elevated in the vehicle-treated heart transplantation group. Thus, pharmacological PARP inhibition reduces reperfusion injury after heart transplantation due to prevention of energy depletion and downregulation of adhesion molecules and exerts a beneficial effect against reperfusion-induced graft coronary endothelial dysfunction. (Circ Res. 2002;90:●●●.●●●.)

Key Words: transplantation ■ reperfusion injury ■ PARP inhibition ■ endothelial function ■ rat

Ischemia/reperfusion injury is a common condition during cardiac surgery. Myocardial performance within the first hours after the surgical procedure determines the patient's state not only during the postoperative period but also in the long-term outcome, especially after heart transplantation when an extended time of ischemia is followed by reperfusion. Most studies about the effects of myocardial ischemia and reperfusion focus on myocardial injury and the recovery of contractile function. It is now appreciated that the survival of the heart as a whole depends in part on the ability of the microcirculation to deliver and distribute blood flow adequately during reperfusion. Recent studies show the importance of protecting the microvasculature to attenuate reperfusion injury.1 Therefore, novel therapeutic strategies concentrate on management modalities that prevent both myocardial and endothelial injury during reperfusion.

Ischemia/reperfusion injury initiates a pathophysiological cascade including an inflammatory response with liberation of cytokines and free radicals. A recently discovered mechanism of cell injury, the poly-ADP-ribose polymerase (PARP) pathway (see Sims et al2 and Schraufstetter et al3; overview in Szabó4) is involved in the pathogenesis of various forms of ischemia/reperfusion injury. In 1997, Thiemermann et al5 and Zingarelli et al6 independently demonstrated that pharmacological inhibition of PARP reduces myocardial necrosis and improves cardiac function in coronary ischemia-reperfusion injury. In addition, the beneficial effects of PARP deficiency7 or PARP inhibitors8 on functional contractile parameters7,8 and on high-energy phosphates8 after global ischemia/reperfusion of the heart have been reported. We surmised that modulation of this pathway may improve early cardiac graft function. 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 the reperfused heart.2–8 PARP activation also strongly upregulates expression of the transcription factor AP-1 and AP-1–dependent genes, including intracellular adhesion molecule (ICAM-1).9 Thus, inhibition of PARP activity prevents energy depletion and granulocyte inflammation. It has been demonstrated in

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vitro in cardiac myoblasts, endothelial cells, and vascular smooth muscle cells that PARP has a significant role in cell injury induced by peroxynitrite, a potent oxidant species produced in various forms of reperfusion.10–13 The use of a PARP inhibitor for the prevention of reperfusion injury in the context of cardiac transplantation has not yet been investigated.

Therefore, the aim of the present study was to test the hypothesis that PARP inhibition with a novel, potent, water-soluble phenanthridione derivative PARP inhibitor, PJ34 (N-(6-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide),14,15 improves myocardial and endothelial function and cardiac morphology during cardiac preservation and reperfusion in our well-established rat model of heterotopic transplantation.16,17

Materials and Methods
Heterotopic Heart Transplantation
The experimental model was described elsewhere (see online data supplement).16,17 Briefly, donor hearts were explanted from Lewis rats. After 1 hour of ischemic preservation at 4°C, the hearts were implanted intraabdominally anastomosing the aorta and the pulmonary artery of the donor heart with the abdominal aorta or the vena cava of the recipient rat, respectively.

All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Functional Measurements in the Graft
Left ventricular systolic pressure (LVSP), end diastolic pressure (LVEDP), rate of pressure development (dP/dt), and relaxation time constant (τ₀) were measured by a Millar micromanometer (Millar Instruments, Inc) at different LV volumes using an intraventricular balloon.16,17 Total coronary blood flow (CBF) was measured by a perivascular ultrasonic flow probe on the donor aorta. After baseline measurement, the endothelium-dependent vasodilator acetylcholine (ACH, 1 nmol/L, 0.2 mL) as well as the endothelium-independent vasodilator sodium nitroprusside (SNP, 10 nmol/L, 0.2 mL) were administered directly into the coronary arteries of the graft via the donor aorta. Between the infusions, CBF was allowed to return to baseline levels. Vasodilator response was expressed as maximum percent change of CBF from baseline.

Histological Analysis
Acetone fixed cryostat sections were stained with hematoxylin-eosin and examined by light microscopy by two independent investigators to limit the influence of observer basis.

PAR Immunohistochemistry
Acetone fixed sections were stained by primary mouse monoclonal anti–poly(ADP-ribose) antibody (Alexis, San Diego, Calif) to detect the product (poly-ADP ribose) of PARP activity (see online data supplement).18

P-Selectin and ICAM-1 Immunohistochemistry
Immunohistological stainings were performed using the APAAP technique described by Mason et al.19 The acetone-fixed cryostat sections were stained with the following primary antibodies: polyclonal rabbit anti-human P-selectin (PharMingen Int, San Diego, Calif, 1:100 dilution), which gives an excellent cross-reaction with rat P-selectin, and mouse anti-rat intracellular adhesion molecule (ICAM-1, Seikagaku America, Falmouth, Mass, 1:100 dilution). Quantitative histomorphological assessment was performed by the COLIM software package (Pictron Ltd) based on the intensity and distribution of labeling. The results were expressed with a grading system of 0 (no staining) to 4 (extensive staining) based on the measured intensity and area of positive labelings (see online data supplement).

Determination of High-Energy Phosphates
Creatine phosphate (CP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) contents were assessed with standard photometry using an enzyme-kinetic assay (see online data supplement). Energy charge potential was calculated as [ATP + 0.5ADP]/[ATP + ADP + AMP].

Experimental Protocol
Four transplant groups were studied (n=6/group). Immediately before releasing the aortic clamp, the slow injection of either saline (control group) or the novel phenanthridione PARP inhibitor PJ34 (3 mg/kg) was started and continued during the first 5 minutes of the reperfusion period. This dose was chosen based on in vitro and in vivo studies (see online data supplement), previous efficacy data with the compound in various models of inflammation and vascular injury,14,15 and pilot transplant experiments. In Group A (control) and B (PJ34), the measurements of systolic and diastolic function and CBF were performed after 1 hour of reperfusion. In Group C (control) and D (PJ34), the abdominal cavity was closed, and the animals were allowed to recover from the anesthesia. During the following 24 hours, the animals of both groups received the same standard diet and normal drinking water. After 24 hours, the animals were reanesthetized and the abdominal cavity was reopened. The grafts were instrumented and the measurements were performed as in Group A and B. After the functional measurements, the hearts were excised for histological analysis.

In a separate series of experiments, 4 groups (n=6/group) of hearts were transplanted and treated with either PJ34 or saline vehicle similarly to the above mentioned protocol. After either 1 or 24 hours of reperfusion, the grafts were excised to determine high energy phosphate contents.

The nature of the model and the protocol above did allow the characterization of temporal changes in heart function during reperfusion; they did not, however, allow an absolute comparison with preischemic values. To address this issue, a modified nonischemic sham-operated transplant group was investigated (Group NI, n=6). Donor hearts were immediately perfused by the recipient via thin polyethylene tubes connected to the donor aorta and pulmonary artery and the abdominal aorta and vena cava of the recipient, respectively, and assessed after 1 hour of perfusion (see online data supplement). In order to confirm that the observed effects of PJ34 were specifically related to PARP inhibition and were unrelated to independent pharmacological actions of the PARP inhibitor compound tested, we have repeated the functional studies using 5-aminosquinolinolone (5-AIQ, 2 mg/kg), a potent PARP inhibitor of a different structural class. The experiments were in all aspects identical to what has been described for PJ34 above (see online data supplement).

Finally, we also wished to confirm that the PARP inhibitors used, PJ34 and 5-AIQ, fail to affect baseline cardiac function in normal animals. These studies were conducted in isolated Langendorff heart preparations, as well as in anesthetized rats using a Millar catheter-based method (see online data supplement).

Statistical Analysis
All values were expressed as mean±standard error of the mean (SEM). Individual means between the groups were compared by one-way analysis of variance followed by an unpaired t test with a Bonferroni correction for multiple comparisons and the post hoc Scheffe’s test. A value of P<0.05 was considered statistically significant.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.
Results

Early Reperfusion, 60 Minutes, and Nonischemic Transplants

The hemodynamic parameters and myocardial blood flow after 60 minutes of reperfusion are shown in Table 1. The recipient’s heart rate and aortic pressure were same in all groups. Systolic functional recovery was significantly better in the PJ34 group in comparison to control. LVSP and peak positive dP/dt were significantly \((P < 0.05)\) higher in the PJ34 group. Systolic cardiac function curves showed a significant leftward shift in the PJ34 group in comparison to the vehicle treated group (Figures 1A and 1B). Peak negative dP/dt was significantly higher \((P < 0.05)\) and TE significantly lower \((P < 0.05)\) in the PJ34 group, indicating a better myocardial relaxation (Table 1). The values of the nonischemic group were significantly higher in comparison to the vehicle treated transplant group; however, there were no differences in comparison to the PJ34-treated transplant group. LVEDP did not differ between the groups. The diastolic compliance curves (end diastolic pressure–volume relationships) were similar in all groups (Figure 1C). CBF was significantly higher \((P < 0.05)\) in the PJ34 group in comparison to control after 60 minutes (Figure 2A). Endothelium-independent vasodilatation after SNP (Figure 2C) was similar in both groups. In contrast, endothelium-dependent vasodilatation after ACH and BK was significantly \((P < 0.05)\) better in the PJ34 group than in the vehicle-treated transplant group (Figure 2B).

In order to confirm that the observed effects of PJ34 were specifically related to PARP inhibition and were unrelated to independent pharmacological actions of the PARP inhibitor compound tested, we have repeated the functional studies using 5-AIQ. The experiments demonstrated that 5-AIQ, similar to PJ34, significantly improved LVSP, peak positive dP/dt, and TE as well as endothelium-dependent vasodilatation after ACH and BK (see online data supplement). Neither PARP inhibitors tested affected baseline myocardial function, as confirmed in isolated hearts as well as in anesthetized animals (see online data supplement).

Myocardial high energy phosphate content, especially ATP-content as well as energy charge potential, were pre-

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Table 1. Functional Parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Recipient</th>
<th>HR, min⁻¹</th>
<th>AoP, mm Hg</th>
</tr>
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<tbody>
<tr>
<td>Group A</td>
<td>289±26</td>
<td>84±8</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>303±20</td>
<td>89±8</td>
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</tr>
<tr>
<td>Group NI</td>
<td>300±27</td>
<td>87±12</td>
<td></td>
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<tr>
<td>Group C</td>
<td>316±35</td>
<td>86±6</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>292±30</td>
<td>92±10</td>
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</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Graft</th>
<th>HR, min⁻¹</th>
<th>LVSP, mm Hg</th>
<th>dP/dtmax, mm Hg/s</th>
<th>dP/dtmin, mm Hg/s</th>
<th>TE, ms</th>
<th>LVEDP, mm Hg</th>
<th>CBF, ml/min/g</th>
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<tbody>
<tr>
<td>Group A</td>
<td>189±33</td>
<td>82±4</td>
<td>1740±116</td>
<td>989±115</td>
<td>12.9±1.5</td>
<td>5.8±1.4</td>
<td>2.86±0.35</td>
<td>4.20±0.56</td>
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<tr>
<td>Group B</td>
<td>176±25</td>
<td>112.9*</td>
<td>3133±609*</td>
<td>2454±461*</td>
<td>9.4±1.1*</td>
<td>7.6±2.5</td>
<td>4.20±0.35</td>
<td>4.20±0.56</td>
</tr>
<tr>
<td>Group NI</td>
<td>288±21*</td>
<td>127±13*</td>
<td>4397±602*</td>
<td>2811±511*</td>
<td>7.0±0.8*</td>
<td>5.2±1.0*</td>
<td>4.48±0.46*</td>
<td>3.94±0.39</td>
</tr>
<tr>
<td>Group C</td>
<td>278±31†</td>
<td>114±3†</td>
<td>4103±237†</td>
<td>2445±129†</td>
<td>7.7±1.6†</td>
<td>5.1±1.2</td>
<td>3.94±0.39</td>
<td>4.52±0.41</td>
</tr>
<tr>
<td>Group D</td>
<td>265±41†</td>
<td>102±11</td>
<td>4280±803</td>
<td>2722±626</td>
<td>6.9±0.9</td>
<td>5.4±1.4</td>
<td>3.94±0.39</td>
<td>4.52±0.41</td>
</tr>
</tbody>
</table>

Group A: control, 60 minutes of reperfusion; Group B: PJ34 treatment, 60 minutes of reperfusion; Group C: control, 24 hours of reperfusion; Group D: PJ34 treatment, 24 hours of reperfusion; Group NI: nonischemic transplants, 60 minutes of perfusion. HR indicates heart rate; AoP, mean aortic pressure; LVSP, left ventricular systolic pressure; dP/dtmax, maximum rate of pressure development; dP/dtmin, minimum rate of pressure development; TE, time constant of monoeponential isovolumetric pressure decay; LVEDP, left ventricular end diastolic pressure. All values are given as mean±SEM at an intraventricular volume of 80 μL.

*P<0.05 vs control transplant; †P<0.05 24 hours vs 60 minutes.

Figure 1. Left ventricular peak systolic pressure (LVSP)–volume (LVV) (A), maximum pressure development (dP/dt max)–LVV (B), and left ventricular end diastolic pressure (LVEDP)–LVV (C) relationships after 1 and 24 hours of reperfusion. All values are given as mean±SEM; *P<0.05 vs other groups.
served by PJ34 treatment during heart transplantation (Table 2). CP content did not show any significant differences between the groups.

Histological findings reveal a slight edema and in some cases a scar inflammatory perivascular infiltrate composed predominantly of polymorphonuclear neutrophils and lymphocytes in the transplanted heart in comparison with the native hearts of the recipients. Immunohistochemical staining showed increased immunoreactivity for poly(ADP-ribose) (PAR) — indicative of enhanced activation of PARP — in the vehicle-treated transplant group. PAR positive staining was observed in the nucleus of the myocytes and in some cases in the cytosol as an indicator of myocyte cell necrosis. Furthermore, endothelial cell nuclei also showed a strong PAR staining (Figure 3). As expected with the current treatment regimen,17 the staining for PAR was absent in the PJ34 group (Figure 3). There was a marked P-selectin and ICAM-1 staining in the vehicle treated transplant group, which was abolished in the PJ34-treated group (Figure 4). The histology score values were 1.78 ± 0.16 versus 0.28 ± 0.02 (P < 0.05) for P-selectin and 2.96 ± 0.11 versus 0.65 ± 0.07 (P < 0.05) for ICAM-1, respectively.

Late Reperfusion, 24 Hours (Group C and D)

After 24 hours of reperfusion, there were no differences in LVSP, peak positive and negative dP/dt, Te, and LVEDP between the vehicle- and the PJ34-treated transplant groups (Table 1). In the vehicle-treated transplant group, all these parameters showed a significant improvement in comparison to the values after 60 minutes of reperfusion (P < 0.05). In the PJ34-treated transplant group, there were no significant differences in comparison to the values of 60 minutes of reperfusion. Systolic cardiac function curves and diastolic compliance curves of the control and the PJ34 group were nearly identical (Figure 1). Baseline CBF was also similar in both groups. After 24 hours, endothelium-dependent vasodilation was significantly increased (P < 0.05) in both groups in comparison to the 60-minute reperfusion values. Endothelium-dependent vasodilation after both ACH and BK was also significantly higher in the PJ34 group in comparison to vehicle-treated transplant group. (Figure 2).

After 24 hours, total adenylate pool showed no significant differences between the groups; however, ATP content was still slightly higher in the PJ34-treated animals without reaching the level of significance (Table 2). CP content did not change (Table 2).

Standard hematoxylin-eosin staining showed intact myocardium. Immunohistochemical staining showed little activation of PARP in the vehicle group and no PAR staining in the PJ34 group (not shown). No P-selectin activity could be detected in either group. In contrast, ICAM-1 staining was elevated in the vehicle-treated transplanted hearts in comparison to the PJ34-treated transplant hearts (Figure 4, score: 2.38 ± 0.32 versus 0.55 ± 0.05, P < 0.05).

**TABLE 2. High Energy Phosphates**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group NI</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP, μmol/g drw</td>
<td>1.86 ± 0.41</td>
<td>5.07 ± 0.82</td>
<td>6.58 ± 1.12</td>
<td>2.65 ± 0.49</td>
<td>4.20 ± 0.66</td>
</tr>
<tr>
<td>ADP, μmol/g drw</td>
<td>2.05 ± 0.42</td>
<td>3.29 ± 0.36</td>
<td>3.48 ± 0.16</td>
<td>3.35 ± 0.55</td>
<td>3.09 ± 0.45†</td>
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<tr>
<td>AMP, μmol/g drw</td>
<td>2.07 ± 0.22</td>
<td>1.72 ± 0.25</td>
<td>1.91 ± 0.22</td>
<td>0.49 ± 0.14†</td>
<td>0.88 ± 0.34†*</td>
</tr>
<tr>
<td>CP, μmol/g drw</td>
<td>6.62 ± 0.78</td>
<td>8.00 ± 1.26</td>
<td>7.94 ± 0.98</td>
<td>6.71 ± 0.66</td>
<td>5.43 ± 0.64</td>
</tr>
<tr>
<td>ECP</td>
<td>0.49 ± 0.04</td>
<td>0.66 ± 0.05*</td>
<td>0.69 ± 0.07*</td>
<td>0.53 ± 0.05</td>
<td>0.61 ± 0.06</td>
</tr>
</tbody>
</table>

Group A: control, 60 minutes of reperfusion; Group B: PJ34 treatment, 60 minutes of reperfusion; Group C: control, 24 hours of reperfusion; Group D: PJ34 treatment, 24 hours of reperfusion; Group NI: nonischemic transplants, 60 minutes of perfusion. ATP indicates adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; CP, creatine phosphate; ECP, energy charge potential; drw, dry weight.

*P < 0.05 vs control; †P < 0.05 24 hours vs 60 minutes.
Discussion

In this study, the benefits of the application of the novel PARP inhibitor PJ34 during reperfusion were assessed after reversible hypothermic ischemia in a heterotopic rat heart transplantation model. Heterotopic heart transplantation was used to simulate the clinical conditions in terms of whole blood reperfusion and to allow an observation time of 24 hours, which is impossible in isolated organ models. Furthermore, the heterotopic situation also allows for the assessment of myocardial function independently from the actual loading conditions. To our best knowledge, this is the first study that describes the cardioprotective effects of a PARP inhibitor in a clinically relevant transplant model. We demonstrated that cardiac preservation (global deep hypothermic ischemia) followed by reperfusion leads to a significant activation of PARP, which was blocked by the novel PARP inhibitor PJ34. Furthermore, the data of the present study show that the activation of PARP is transient, as after 24 hours of reperfusion very little PARP activity was detected in the vehicle-treated control animals. The data show that the inhibition of the PARP pathway improves myocardial and endothelial functional recovery during early reperfusion and attenuates energy depletion and the activation of adhesion molecules.

Figure 3. Immunohistological staining against poly-ADP-ribose, a marker of poly(ADP-ribose) polymerase activation after 1 hour of reperfusion. Top panels, low magnification (100×); bottom panels, high magnification (400×). The control specimens (left panels, A and C) showed positive staining in the nuclei of the myocytes and in the capillary endothelium. The PJ34 group (right panels, B and D) showed completely negative staining. Arrows indicate positive staining in the control group.

Figure 4. Immunohistological staining against P-selectin and ICAM-1. Top row, Staining against P-selectin in the control (A) and in the PJ34-treated (B) group after 1 hour of reperfusion. Middle row, ICAM-1 staining in the control (C) and in the PJ34-treated (D) group after 1 hour of reperfusion. Bottom row, ICAM-1 staining in the control (E) and in the PJ34-treated (F) group after 24 hours of reperfusion. Arrows indicate positive staining (activation of P-selectin or ICAM-1, respectively) in the control group.
Furthermore, the initial treatment with PJ34 has a persisting long-term beneficial effect on endothelial function.

To ensure that the observed effects are specific to PARP inhibition, we repeated the functional measurements and high-energy phosphate determinations with a second PARP inhibitor, 5-AIQ, with nearly identical results. Furthermore, we performed pharmacokinetic and hemodynamic measurements, as well as dose-response experiments in vivo and in isolated hearts. In a series of control experiments, we have confirmed that the compound does not exert direct cardiac effects in normal (nontransplanted) hearts. We found that PJ34 (or 5-AIQ) did not affect cardiac function in the therapeutically relevant dose and concentration range. Thus, the improved cardiac function seen in the transplanted hearts is a specific phenomenon, i.e., prevention of the cardiac suppression due to ischemic and reperfusion injury, rather than the consequence of some nonspecific cardiotoxic effect of PARP inhibition. Furthermore, the effects are not specific for PJ34 but represent a general feature for pharmacological PARP inhibition (data shown in online supplement).

The activation of PARP is currently described to be a final common effector in various types of tissue injury, including systemic inflammation, circulatory shock, and ischemia/reperfusion (see Introduction). The genetic disruption of the PARP pathway effectively protects against oxygen radical and nitric oxide toxicity in different cell types, such as in pancreatic islet cells and in thymocytes, and attenuates regional myocardial ischemia/reperfusion and global hypoxia-reoxygenation injury. Furthermore, the pharmacological blockade of PARP also results in a protection against peroxynitrite injury in cardiomyocytes, endothelial cells, and fibroblasts and reduces myocardial infarct size in a regional ischemia model. 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 ischemia/reperfusion. There are multiple mechanisms of the protective action of PJ34. Comparing the data with nons ischemic transplants cardiac preservation/reperfusion injury leads to a significant decrease of high energy phosphate content in control grafts. Previous and the present data clearly demonstrate that the prevention of PARP activation by PJ34 results in a better preservation of the total adenylate pool, primarily by the increased myocardial ATP content, resulting in an improved energy status as expressed by the significantly higher energy charge potential. It has been shown previously that intermediate periods of ischemia induce a severe loss of cellular NAD⁺ and ATP levels. The loss of cellular energetic pools, in turn, importantly affects myocardial function. Based on the results of the present study, we propose that the inhibition of PARP by PJ34 during reperfusion may contribute to a better recovery of the cellular ATP and thereby improve myocardial contractility and relaxation. Moreover, it was shown that energy depletion mediated by PARP after oxidant stress significantly contributes to endothelial injury in cultured pulmonary artery endothelial cells, in endotoxin shock, and also in diabetes mellitus in vivo. Thus, improved endothelial function can also be explained at least partly by the improved energetic balance of the endothelium.

CP contents showed no significant differences between the groups including nonischemic transplants. It would indicate that either the ischemic stimulus was too low to induce degradation of CP pools or CP pools regenerate during the reperfusion phase. Galinanes et al showed in a similar transplant model that CP contents are significantly reduced after 1 hour of ischemic preservation and completely restored after 1 hour of reperfusion.

We also showed that PARP activation contributes to the expression of P-selectin and ICAM-1 in global hypothermic cardiac ischemia/reperfusion and consequently to the recruitment of neutrophils into jeopardized tissue. This finding is consistent with our previous reports in different models of regional ischemia/reperfusion. During the early phase of reperfusion, P-selectin is rapidly released to the cell surface from preformed pools after exposure to a certain stimuli and allows to roll along the endothelium. ICAM-1 constitutively expressed on the surface of endothelial cells is then involved in neutrophil adhesion. A significant upregulation of ICAM-1 was demonstrated under ischemia/reperfusion with a parallel increase of neutrophil activity. We have previously reported that genetic disruption of PARP abolished the expression of P-selectin and the upregulation of ICAM-1, while maintaining unaffected the constitutive levels of ICAM-1 on endothelial cells after 1 hour of coronary occlusion and 1 hour of reperfusion in a mouse model. The results of the present study clearly demonstrate that the inhibition of PARP activity can interrupt the interaction between neutrophils and endothelial cells, both at the early rolling phase mediated by P-selectin and at the late firm adhesion phase mediated by ICAM-1.

This is the first study in which long-term effects of a PARP inhibitor, PJ34, on global hypothermic ischemia/reperfusion injury were investigated. Comparing the data after 1 and 24 hours reperfusion as well as the data of nonischemic transplants, we can conclude that the administration of PJ34 in the given dose was able to completely prevent reperfusion injury after a short period of hypothermic preservation. On the other hand, the control group showed a recovery after 24 hours with similar values to the PJ34 group, suggesting that the applied cardiac preservation time and reperfusion lead to only reversible changes of functional status of the heart.

Although no differences were found between the groups in systolic and diastolic function and baseline coronary blood flow after 24 hours of reperfusion, endothelial function was still depressed in the control group as indicated by the lower CBF response to acetylcholine and bradykinin. The fact that after 24 hours reperfusion the histological specimens from both control and PJ34 showed only little PARP activation suggested that delayed endothelial dysfunction in the control animals may be a late consequence of a transient, earlier burst of PARP activation and subsequent cellular alterations. Inhibition of PARP prevents energy depletion of endothelial cells and thereby improves endothelial function and preserves endothelial cell integrity, which has prolonged cellular protective effects. Furthermore, the prolonged up-regulation of ICAM-1 in the control group and the absence of
such ICAM-1 upregulation after PI34 treatment indicate that prolonged upregulation of certain adhesion molecules are at least partly responsible for the delayed endothelial dysfunction.

We have compared the data with previous rat heart transplant studies with similar ischemia/reperfusion protocols. Summarizing the data of these studies, a biphasic recovery pattern is characteristic for this model with an early phase (<1 hour) followed by a further improvement during the next 24 hours. Furthermore, different agents such as adenosine, endothelin receptor antagonists, the NO-precursor l-arginine, the NO-donor SIN-1 (unpublished data), and free radical scavengers effectively reduced reperfusion injury. Taking into account that in some of the studies ischemic time or assessment protocol was different, PARP inhibition seems to be an effective therapeutic modality for reducing reperfusion injury in comparison to other, previously tested agents.

Beside mechanical function and coronary flow, we assessed endothelial function in our previous and in the present experiments. We showed a slower recovery of endothelial function, which indicates that the coronary endothelium is more vulnerable to reperfusion injury than the myocardium. Indeed, Mizuno et al demonstrated that after normothermic ischemia and reperfusion, myocardial and endothelial function can be dissociated: although myocardial function showed a full recovery in their model, endothelial function remained impaired. Schnabel et al showed in a recent ultrastructural study in human transplant biopsy specimens that whereas myocyte ultrastructural integrity recovers within 60 minutes of reperfusion, ultrastructural regeneration of the endothelium lasts from days up to 1 week.

Beside the improvement of energetic balance and the structure of the endothelium, PARP inhibition in the early reperfusion phase may have additional effects that influence the long-term course of endothelial function or otherwise initial PARP activation may initiate processes that are responsible for persistent endothelial dysfunction in the control animals. It has been demonstrated that the inhibition of PARP improves mitochondrial respiration in cardiac myocytes and endothelial cells in the setting of peroxynitrite injury. It is also known that, in addition to the energetic changes, poly(ADP-ribosyl)ation may lead to the relaxation of chromatin, with the consequence that genes become more accessible to RNA polymerase. PARP regulates expression of variety of genes including inducible NO synthetase, collagenase, and ICAM-1. PARP has been shown to regulate, directly or indirectly, promoter activation: in human endothelial cells, inhibition of PARP reduces oxidant induced binding activity of the transcription factor activator protein-1 to the promoter of ICAM-1. The significance of PARP-induced modification of gene expression was not in the scope of the present study; however, it is conceivable that such changes may also contribute to the prolonged endothelial dysfunction in the vehicle-treated transplant group in the present study.

In summary, we have demonstrated that hypothermic cardiac preservation followed by reperfusion results in an activation of PARP, which, in turn, leads to significantly reduced recovery of myocardial and endothelial function. We have also shown that PARP activation contributes to reperfusion injury by 2 different mechanisms: (1) significant depletion of cardiac energy stores and (2) activation of adhesion molecules. Furthermore, the initial activation of PARP contributes to a prolonged endothelial dysfunction after transplantation. In the present study, potent PARP inhibitors were able to markedly attenuate transplant reperfusion injury. Additional preclinical and eventual clinical studies with PARP inhibitors are warranted to reduce reperfusion injury and improve graft quality in various models of cardiac transplantation.

Acknowledgments

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References


Poly(ADP-Ribose) Polymerase Inhibition Reduces Reperfusion Injury After Heart Transplantation


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1. Expanded Material and Methods

Heterotopic heart transplantation

The donor rat was anaesthetized with intraperitoneal xylazine (7 mg/kg). After the abdominal cavity was opened 500 units of sodium heparin were injected into the inferior vena cava. Then bilateral thoracotomy was performed and the heart was exposed. The superior and inferior vena cava and the pulmonary veins were tied en masse with a 4-0 single silk suture. The aorta and the pulmonary artery were divided and the heart was immediately placed into cold saline (4 °C). The donor organ was prepared within five minutes.

The recipient rats were anaesthetized with intraperitoneal xylazine (7 mg/kg) and heparinized with 500 IU heparin intravenously. The abdomen was opened by a midline incision and the aorta and the vena cava were exposed by reflecting the intestines to the left side. Two-centimeter segments of the infrarenal aorta and the vena cava were isolated and occluded by small vessel forceps. The aorta and the pulmonary artery of the donor heart were anastomosed end to side to the abdominal aorta or the vena cava of the recipient rat, respectively. This was achieved using a 9-0 monofilament polyamide sutures operating under a 16-power magnification microscope. The heart was maintained at 6-9 °C during the implantation period by wrapping it in cold gauze which was regularly irrigated with cold saline (4 °C). To minimize variability between experiments, the duration of the implantation was standardized at 60 minutes for all studies. After completion of the anastomoses, the vessels were released slowly and the heart was then reperfused with blood in situ. Aortic pressure of the recipient was recorded via the femoral artery. Crystalloid volume substitution (Ringer’s solution) was adjusted via the external jugular
vein to maintain mean arterial pressure between 75-85 mmHg during the functional measurements.

“Non-ischemic” transplants

A modified technique of intraabdominal transplantation was applied which allows quick implantation of the donor heart without a significant ischemic time. Donor and recipient rats were anesthetized as described above. First, recipients were prepared. The animals were heparinized with 500 IU heparin intravenously. The abdomen was opened by a midline incision and the aorta and the vena cava were exposed by reflecting the intestines to the left side. Two-centimeter segments of the infrarenal aorta and the vena cava were isolated and occluded by small vessel forceps. Thin polyethylene tubes were inserted via a small incision into the abdominal aorta and inferior vena cava, respectively. The connections were tightened by local application of tissue glue. Afterwards, the donor hearts were explanted as described above. The donor aorta and pulmonary artery were attached immediately to the corresponding aortic and caval tubes and fixed with 5-0 single silk sutures. Then, the recipient vessels were released and the heart was perfused with blood for a one-hour period.
Functional measurements in the graft

After 60 minutes and 24 hours of reperfusion, respectively, a latex balloon was introduced into the left ventricle (LV) via the apex and was connected to a precision calibrated syringe for administration and withdrawal of fluid to determine LV pressure, its first derivative (dP/dt) and enddiastolic pressure (LVEDP) by a Millar micromanometer (Millar Instruments, Inc., Houston, Tex) at different LV volumes. From these data LV pressure-volume relationships were constructed. Myocardial relaxation was characterized by the relaxation time constant ($T_f$) of the monoexponential decay of LV pressure fall. Total coronary blood flow (CBF) was measured by a perivascular ultrasonic flow probe on the donor aorta. After baseline measurement, the endothelium-dependent vasodilator acetylcholine (ACH, 1 nM, 0.2 ml) and bradykinin (BK 0.1 nM, 0.2 ml) as well as the endothelium-independent vasodilator sodium-nitroprusside (SNP, 10 nM, 0.2 ml), respectively, were administered directly into the coronary arteries of the graft via the donor aorta. Between the infusions, CBF was allowed to return to baseline levels. Vasodilator response was expressed as the maximum percent change of CBF from baseline.

Histologic analysis

After the functional measurements the hearts were excised, immediately immersed in fluid nitrogen (-196 °C), and stored at -80 °C. Later transverse sections were fixed in acetone at -20°C for 10 minutes and after washing in Tris-buffered solution (TBS), pH7.6 for 2x7 minutes, stained with hematoxylin-eosin for examination by light microscopy. Six hematoxylin-eosin
sections per heart were examined by two independent investigators to limit the influence of observer basis.

PAR immunohistochemistry The acetone-fixed cryostat sections were treated with 0.3% hydrogen peroxide for 15 min to eliminate endogenous peroxidase activity and then rinsed briefly in 10 mM PBS. Non-specific binding was blocked by incubating the slides for 1h in TBS containing 2% horse serum. Mouse monoclonal anti-poly (ADP-ribose) antibody (Alexis, San Diego, CA) and isotype-matched control antibody were applied in a dilution of 1:100 for 2h at room temperature. Following extensive washing (5 X 5 min) with TBS, immunoreactivity was detected with a biotinylated goat anti-rabbit secondary antibody and the avidin-biotin-peroxidase complex (ABC) both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, CA). Color was developed using Ni-DAB substrate (95 mg diaminobenzidine, 1.6 g NaCl, 2 g NiSO₄ in 200 ml 0.1M acetate buffer). Sections were then counterstained with nuclear fast red, dehydrated and mounted in Permount.

P-selectin and ICAM-1 immunohistochemistry. Immunohistological stainings were performed using the APAAP technique. The acetone-fixed cryostat sections were incubated for 60 minutes with the primary antibody (see below) followed by incubation with bridging biotinylated anti-mouse (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) or anti-rabbit (BioGenex Laboratories Inc., San Ramon, CA) antibody for 30 minutes respectively. After 30-minute incubation with label alkaline phosphatase and 20-minute incubation with levamisole (0.8 g/l) the specimens were counterstained with hematoxylin and mounted. Between all steps of the procedure the sections were washed with TBS for 3x5 minutes. The following primary antibodies were used: polyclonal rabbit-anti-human P-selectin (PharMingen Int., San Diego, CA,
1:100 dilution) which gives an excellent cross-reaction with rat P-selectin, and mouse-anti-rat intracellular adhesion molecule (ICAM-1, Seikagaku America, Falmouth, MA, 1:100 dilution).

Immunohistologic stainings were evaluated by the COLIM software package (Pictron Ltd., Budapest, Hungary). A digital camera was used to input microscopic pictures from a low power (16-40x) magnification of the whole section and a high power (400x) examination of 20 adjacent fields. First, positively stained areas were separated from each other and from the background based on the intensity of different colors of the specimen. The colours found in the object were put into 5 color classes: one class for background staining, and four classes for positively stained areas. On the base of measured intensity, the colour classes were coupled with score values. as follows: 0, no positive staining, 1 to 3 increasing degrees of intermediate staining; and 4, extensive staining. It is noteworthy, that in the case of ICAM-1 minimal staining could be found even in normal non-ischemic hearts due to constitutive presence of staining along the endothelial wall. The program automatically measured the area of the objects in each class in each field and calculated an average score for the whole picture. Finally, each specimen was characterized with the average of the 20 adjacent fields.

Determination of high energy phosphates

After excision the hearts were immediately immersed in fluid nitrogen (-196 °C) and stored frozen at -80 °C until the biochemical measurements. One gram heart tissue was homogenized in 10 ml 3.5% HClO₄ and than centrifuged with 20,000 U/min at 5 °C. Five milliliter supernatant was neutralized with 1ml Triethanolamin-HCL/K₂CO₃ solution. Creatine phosphate (CP) and adenosine triphosphate (ATP) degradation was assessed with standard
photometry using the enzyme kinetic assay containing glycerinaldehyd-3-phosphate dehydrogenase, 3-phosphoglycerat-kinase, glycerin-3-phosphat dehydrogenase and triosephosphate-isomerase. CP, ATP, adenosine diphosphate (ADP) and adenosine monophosphate (AMP) contents were expressed as [μmol/g dry weight]. The values obtained were used to calculate energy charge potential as [ATP+0.5ADP] / [ATP+ADP+AMP].

Materials

All materials used in our study was purchased from Sigma-Aldrich (St Louis, MO), unless specified otherwise. PJ34 (N-(6-oxo-5,6-dihydro-phenanthridin-2-yl) -N,N-dimethylacetamide) was synthesized at Inotek Corporation.

2. Comparison of the effects of PJ34 with another PARP-inhibitor 5-AIQ

To ensure that the observed effects are specific to PARP-inhibition we repeated the functional measurements and high energy phosphate determinations with a second PARP inhibitor, 5-AIQ. Online Figure 1 shows that at a dose of 2 mg/kg, 5-AIQ leads to a similar improvement of myocardial contractility and relaxation and coronary blood flow. Furthermore, endothelium-dependent and -independent vasoresponses were also comparable with the PJ34 group (Online Figure 2). Treatment with both PJ34 and 5-AIQ resulted in a significantly better preservation of ATP contents in comparison to control grafts after one hour of reperfusion (5.07±0.82 and 5.56±071, respectively vs. 1.86±0.41 μmol/g dry weight, p<0.05). The fact that the alternative
PARP-inhibitor 5-AIQ achieves the same effects as PJ34 indicates that the described beneficial influence of PJ34 in the setting of hypothermic ischemia/reperfusion can be attributed to PARP-inhibition.

**Legends**

Online Figure 1. Left ventricular systolic pressure (LVSP, A), maximum rate of pressure development (dP/dt\(_\text{max}\), B), isovolumic relaxation constant (T\(_E\), C) and coronary blood flow (CBF, D). All values are given as mean±SEM. *p<0.05 PJ34 or 5-AIQ vs. control at a given time point, †p<0.05 24 hours vs. 60 minutes.

Online Figure 2. Vasodilator response after application of the endothelium-dependent vasodilator bradykinin (0.1 nM, A) and acetylcholine (1 nM, B) and the endothelium independent vasodilator sodium nitroprusside (10 nM, C). All values are given as mean±SEM. *p<0.05 PJ34 or 5-AIQ vs. control at a given time point, †p<0.05 24 hours vs. 60 minutes.

3. **In vitro and in vivo effects of PJ34 and 5-AIQ in normal hearts**

**Methods**

*Pharmacokinetic measurements.*

Rats were injected with PJ34 i.v. at time 0. Plasma samples were taken and analyzed by HLPC for PJ34 concentrations at 5, 10, 30, 60, 120, 240 and 360 minutes.
Cardiovascular determinations

Langendorff heart perfusions

Hearts were rapidly excised from pentobarbital-anesthetized rats and briefly rinsed by immersion into ice-cold Krebs-Henseleit buffer. Spontaneously beating hearts were perfused via the aorta at a constant pressure of 80 mmHg with Krebs-Henseleit buffer at 37°C. A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle through an incision in the left atrium and through the mitral valve, and the volume was adjusted to achieve an end-diastolic pressure of 5 to 8 mmHg. Heart rate, coronary flow and left ventricular pressure were monitored as described [1]. The rate pressure product (RPP) was calculated as the product of heart rate and left ventricular developed (systolic minus end-diastolic) pressure. After 15 min equilibration separate group of hearts were subjected to cumulatively increasing concentrations of either PJ-34 or 5-AIQ. The compounds were infused to the aortic perfusion line at a rate giving either 1, 3, 10 and 30 μM final concentration in the perfusate. The hearts were perfused for 10 minutes at each concentration.

Hemodynamic measurements in rats

Animals were treated with PJ-34 or 5-AIQ at 10mg/kg day i.p., at time 0. The same treatment was repeated 12 hours later, and hemodynamic measurements were performed 3 hours subsequent to the second injection. In preliminary studies (data not shown), the effect of acute i.v. injection of the inhibitors, or a chronic treatment with PJ34 for 4 weeks at 10 mg/kg/day were also tested. None of these treatments exerted any significant effects on cardiac function.

Analysis of left ventricular performance was measured after i.p. injection of thiopentone sodium (60 mg/kg) as described previously [2]. Animals were placed on controlled heating pads, and core temperature measured via a rectal probe was maintained at 36-38 °C. A microtip catheter transducer (SPR-524; Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle under pressure control. After stabilization for 15-20 min, the pressure signal was continuously recorded using a MacLab A/D converter (AD Instruments, Mountain View, CA), and stored and displayed on an Apple Macintosh personal
computer. The heart rate, the left ventricular systolic and end-diastolic pressures were measured and the maximal slope of systolic pressure increment (+dP/dt) and diastolic pressure decrement (-dP/dt), an indexes of contractility and relaxation, were calculated. After these measurements, the catheter was pulled back into the aorta for the measurement of arterial blood pressure. After the hemodynamic measurements were made, animals were sacrificed by lethal injection of thiopentone sodium.
Results

Pharmacokinetic measurements

Intravenous injection of 10 mg/kg PJ34 produced plasma concentrations of 2.1±0.3 μM at 5 minutes after injection. By 1 hour, plasma concentrations fell to 0.8±0.2 μM, and by 2 hours plasma concentrations decreased to 0.2±0.1 μM. At 6 hours post-injection, 62±14 nM concentration was detectable in the plasma.

Cardiovascular measurements

In the in vitro preparation, increasing concentrations of PJ-34 or 5-AIQ did not affect any of the cardiac functions in control animals in the therapeutically relevant concentration range of 1-10 μM. At 30 μM, which is a supratherapeutic concentration, PJ34, but not 5-AIQ exerted slight, but significant cardiodepressant actions (Online Table 1).

In the in vivo experiments, systemic exposure of the animals to PJ34 or 5-AIQ at 10 mg/kg failed to affect any of the cardiovascular parameters studied (Online Table 2).

Discussion

PJ34 was found to be present in the plasma in the peak concentration range of 2 μM, which decreased, over 2 hours, to 200 nM. At 6 hours post-injection, 62±14 nM concentration was detectable in the plasma. Although plasma concentrations do not accurately reflect tissue or cell concentrations, we believe that these levels provided useful pointers towards designing the in vitro studies in perfused hearts, where the concentration range of 0.1-10 μM was considered therapeutically relevant. It is noteworthy that PJ34 is a very potent compound. In cell-free PARP assay, using NAD⁺ and purified PARP-1 enzyme, PJ34 inhibited PARP activity in a dose-dependent manner, with an EC50 of 20 nM. EC50 of the prototypical PARP inhibitor 3-aminoazobenzamide was 200 μM in the same assay [3]. Furthermore, in isolated thymocytes, PJ34
dose-dependently restored peroxynitrite-induced necrosis, with an EC50 of approximately 40 nM [4]. This high potency of the compound is consistent with our immunohistochemical observations showing that PJ34 treatment induced a complete abolishment of poly(ADP-ribose) formation in the myocardial tissue, indicative of in vivo blockade of the enzyme PARP. Similar dosing regimen with PJ34 was previously found to abolish vascular poly(ADP-ribose) formation in diabetes [3] and pulmonary poly(ADP-ribose) formation in endotoxic shock [5].

In a series of control experiments, we have confirmed that the compound does not exert direct cardiac effects in normal (non-transplanted) hearts. We found that PJ34 (or 5-AIQ) did not affect cardiac function in the therapeutically relevant dose and concentration range. Thus, the improved cardiac function seen in the transplanted hearts is a specific phenomenon, i.e. prevention of the cardiac suppression due to ischemic and reperfusion injury, rather than the consequence of some non-specific cardiotonic effect of PARP inhibition.

References


### Online Table 1. Effects of PARS-inhibition in isolated hearts

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>CBF (ml/min)</th>
<th>LVDP (Hgmm)</th>
<th>dP/dt&lt;sub&gt;max&lt;/sub&gt; (Hgmm/min)</th>
<th>dP/dt&lt;sub&gt;min&lt;/sub&gt; (Hgmm/min)</th>
<th>RPP</th>
<th>N</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>250 ± 8</td>
<td>14.1 ± 0.2</td>
<td>85.5 ± 10.3</td>
<td>2355 ± 381</td>
<td>1726 ± 236</td>
<td>21.6 ± 3.3</td>
<td>4</td>
</tr>
<tr>
<td>1 μM PJ34</td>
<td>241 ± 9</td>
<td>13.9 ± 0.4</td>
<td>83.5 ± 10.5</td>
<td>2271 ± 323</td>
<td>1711 ± 287</td>
<td>20.3 ± 3.3</td>
<td>4</td>
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<td>3 μM PJ34</td>
<td>229 ± 9</td>
<td>13.0 ± 0.2</td>
<td>81.5 ± 10.9</td>
<td>2195 ± 322</td>
<td>1620 ± 290</td>
<td>18.9 ± 3.4</td>
<td>4</td>
</tr>
<tr>
<td>10 μM PJ34</td>
<td>252 ± 26</td>
<td>15.2 ± 0.6</td>
<td>78.0 ± 10.3</td>
<td>2178 ± 354</td>
<td>1610 ± 328</td>
<td>20.5 ± 5.1</td>
<td>4</td>
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<tr>
<td>30 μM PJ34</td>
<td>239 ± 16</td>
<td>14.4 ± 0.4</td>
<td>63.8 ± 5.8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>1719 ± 171&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1215 ± 169&lt;sup&gt;*&lt;/sup&gt;</td>
<td>15.5 ± 2.5&lt;sup&gt;*&lt;/sup&gt;</td>
<td>4</td>
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<tr>
<td>Baseline</td>
<td>254 ± 10</td>
<td>13.4 ± 0.5</td>
<td>85.9 ± 5.5</td>
<td>2480 ± 157</td>
<td>2004 ± 114</td>
<td>21.9 ± 1.8</td>
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<tr>
<td>1 μM 5-AIQ</td>
<td>243 ± 9</td>
<td>12.7 ± 0.5</td>
<td>84.9 ± 4.9</td>
<td>2436 ± 149</td>
<td>1955 ± 90</td>
<td>20.6 ± 1.5</td>
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<tr>
<td>3 μM 5-AIQ</td>
<td>230 ± 11</td>
<td>13.2 ± 0.8</td>
<td>81.3 ± 7.4</td>
<td>2271 ± 258</td>
<td>1778 ± 75</td>
<td>18.9 ± 0.6</td>
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<td>10 μM 5-AIQ</td>
<td>244 ± 11</td>
<td>12.9 ± 0.4</td>
<td>84.0 ± 4.6</td>
<td>2439 ± 179</td>
<td>1928 ± 118</td>
<td>20.6 ± 1.7</td>
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<tr>
<td>30 μM 5-AIQ</td>
<td>220 ± 25</td>
<td>13.2 ± 0.5</td>
<td>83.3 ± 7.6</td>
<td>2238 ± 367</td>
<td>1670 ± 106</td>
<td>18.1 ± 0.8</td>
<td>3</td>
</tr>
</tbody>
</table>

HR, heart rate; AoP, mean aortic pressure; LVDP, left ventricular developed pressure; dP/dt<sub>max</sub>, maximum rate of pressure development; dP/dt<sub>min</sub>, minimum rate of pressure development; RPP, rate-pressure product. All values are given as mean±SEM.

<sup>*</sup>p<0.05 vs. baseline
<table>
<thead>
<tr>
<th></th>
<th>MBP (Hgmm)</th>
<th>LVSP (Hgmm)</th>
<th>LVEDP (Hgmm)</th>
<th>dP/dt_{min} (Hgmm/min)</th>
<th>dP/dt_{max} (Hgmm/min)</th>
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<tbody>
<tr>
<td>Control</td>
<td>107.78 ± 7.92</td>
<td>126.43 ± 3.87</td>
<td>5.37 ± 0.24</td>
<td>5772.40 ± 211.13</td>
<td>5013.04 ± 258.74</td>
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<td>P134</td>
<td>108.87 ± 4.80</td>
<td>124.52 ± 4.88</td>
<td>5.59 ± 0.62</td>
<td>5740.62 ± 241.77</td>
<td>5173.44 ± 275.88</td>
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<tr>
<td>SAIQ</td>
<td>109.95 ± 7.06</td>
<td>128.36 ± 4.76</td>
<td>5.68 ± 0.69</td>
<td>5553.28 ± 316.99</td>
<td>5021.75 ± 268.77</td>
</tr>
</tbody>
</table>

HR, heart rate; AoP, mean aortic pressure; LVSP, left ventricular systolic pressure; dP/dt_{max}, maximum rate of pressure development; dP/dt_{min}, minimum rate of pressure development. All values are given as mean±SEM.