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

Gábor Szabó, Susanne Bährle, Nicole Stumpf, Karin Sonnenberg, Éva Szabó, Pál Pacher, Tamás Csont, Richard Schulz, Thomas J. Dengler, Lucas Liaudet, Prakash G. Jagtap, Garry J. Southan, Christian F. Vahl, Siegfried Hagl, Csaba Szabó

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;90:100-106.)**

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 al[2] and Schraufstter et al[3]; overview in Szabó[4]) is involved in the pathogenesis of various forms of ischemia/reperfusion injury. In 1997, Thiemermann et al[5] and Zingarelli et al[6] 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 deficiency[7] or PARP inhibitors[8] on functional contractile parameters[7,8] and on high-energy phosphates[8] 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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From the Departments of Cardiac Surgery (G.S., N.S., K.S., C.F.V., S.H.) and Cardiology (S.B., T.J.D.), University of Heidelberg, Germany; Inotek Corporation (E.S., P.P., L.L., G.J.S., C.S.), Beverly, Mass; and the Cardiovascular Research Group, Department of Pharmacology (T.C., R.S.), Heritage Medical Research Center, University of Alberta, Edmonton, Alberta, Canada.

Correspondence to Gábor Szabó, MD, PhD, Department of Cardiac Surgery, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany. E-mail dzsi@hotmail.com

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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 phenanthridinone 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 (T_E) 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) and bradykinin (BK 0.1 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/each group). Immediately before releasing the aortic clamp, the slow injection of either saline (control group) or the novel phenanthridinone 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/each 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-aminoisoquinolinone (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.

TABLE 1. Functional Parameters

	Group A	Group B	Group NI	Group C	Group D
Recipient					
HR, min^{-1}	289±26	303±20	300±27	316±35	292±30
AoP, mm Hg	84±8	89±8	87±12	86±6	92±10
Graft					
HR, min^{-1}	189±33	176±25	288±21*	278±31†	265±41†
LVSP, mm Hg	82±4	112±9*	127±13*	114±3†	102±11
dP/dt_{max}, mm Hg/s	1740±116	3133±609*	4397±602*	4103±237†	4280±803
dP/dt_{min}, mm Hg/s	989±115	2454±461*	2811±511*	2445±129†	2722±626
T_E, ms	12.9±1.5	9.4±1.1*	7.0±0.8*	7.7±1.6†	6.9±0.9
LVEDP, mm Hg	5.8±1.4	7.6±2.5	5.2±1.0*	5.1±1.2	5.4±1.4
CBF, mL/min/g	2.86±0.35	4.20±0.56	4.48±0.46*	3.94±0.39	4.52±0.41

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/dt_{max}, maximum rate of pressure development; dP/dt_{min}, minimum rate of pressure development; T_E, time constant of monoexponential 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.

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 T_E 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

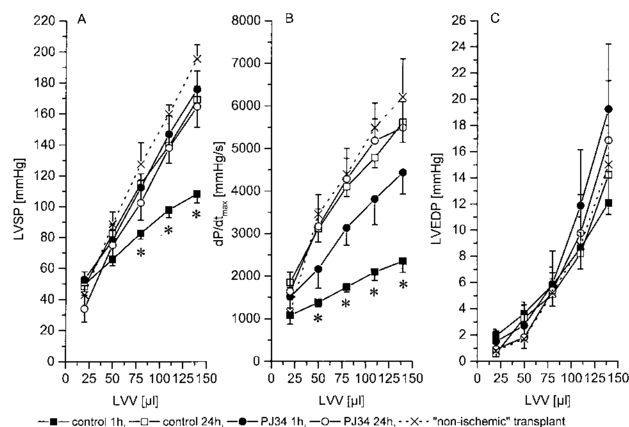

Figure 1. Left ventricular peak systolic pressure (LVSP)–volume (LVV) (A), maximum pressure development (dP/dt_{max})–LVV (B), and left ventricular end diastolic pressure (LVEDP)–LVV (C) relationships after 1 and 24 hours of reperfusion. All values are given as mean±SEM; *$P<0.05$ vs other groups.

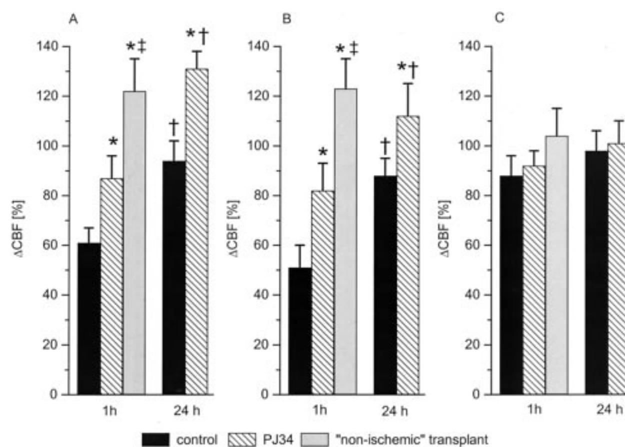

Figure 2. Vasodilator response after application of the endothelium-dependent vasodilator bradykinin (0.1 nmol/L, A) and acetylcholine (1 nmol/L, B) and the endothelium-independent vasodilator sodium nitroprusside (10 nmol/L, C). All values are given as mean±SEM. *$P<0.05$ other groups vs control at a given time point; †$P<0.05$ 24 hours vs 60 minutes; ‡$P<0.05$ nonischemic vs PJ34.

TABLE 2. High Energy Phosphates

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

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.

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 T_E 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 preserved 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 scare 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

After 24 hours of reperfusion, there were no differences in LVSP, peak positive and negative dP/dt, T_E, 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 vasodilatation was significantly increased ($P<0.05$) in both groups in comparison to the 60-minute reperfusion values. Endothelium-dependent vasodilatation 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, $P<0.05$).

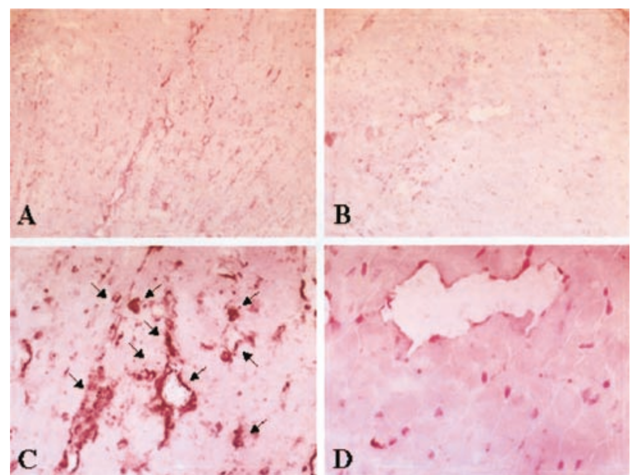

Figure 3. Immunohistological staining against poly-ADP-ribose, a marker of poly(ADP-ribose) polymerase activation after 1 hour of reperfusion. Top panels, low magnification (100×); bottom panels, high magnification (400×). The control specimens (left panels, A and C) showed positive staining in the nuclei 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.

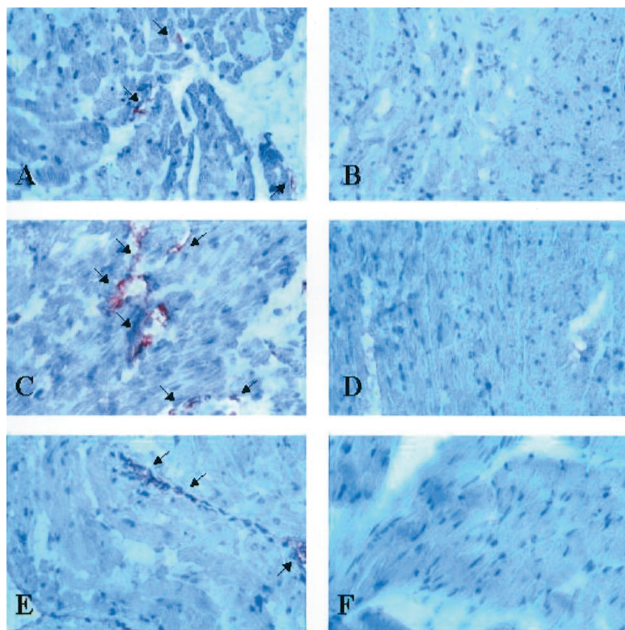

Figure 4. Immunohistological staining against P-selectin and ICAM-1. Top row, Staining against P-selectin in the control (A) and in the PJ34-treated (B) group after 1 hour of reperfusion. Middle row, ICAM-1 staining in the control (C) and in the PJ34-treated (D) group after 1 hour of reperfusion. Bottom 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.

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. 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, ie, prevention of the cardiac suppression due to ischemic and reperfusion injury, rather than the consequence of some nonspecific cardiotonic 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 cultures, such as in pancreatic islet cells[20] and in thymocytes,[12] and attenuates regional myocardial[9] ischemia/reperfusion and global hypoxia-reoxygenation injury.[10] Furthermore, the pharmacological blockade of PARP also results in a protection against peroxynitrite injury in cardiomyocytes,[21] endothelial cells, and fibroblasts[9,13] and reduces myocardial infarct size in a regional ischemia model.[5,6,18] It was also demonstrated that PARP inhibition leads to a significant improvement of endothelial function ex vivo in peroxynitrite-treated thoracic aortic rings[22] and in isolated mesenteric arteries in the setting of splanchnic ischemia/reperfusion.[23]

There are multiple mechanisms of the protective action of PJ34. Comparing the data with nonischemic transplants cardiac preservation/reperfusion injury leads to a significant decrease of high energy phosphate content in control grafts. Previous[8] 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.[7,24] 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.[14,25,26] 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[27] 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.[9,23] 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.[28,29] 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.[28,29] We have previously reported[9] 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 that the applied cardiac preservation time and reperfusion lead to only reversible changes of functional status of the heart.[16,17]

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[14,22] and thereby improves endothelial function and preserves endothelial cell integrity, which has prolonged cellular protective effects. Furthermore, the prolonged upregulation of ICAM-1 in the control group and the absence of such ICAM-1 upregulation after PJ34 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.[16,17,27,30,31] 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,[30] endothelin receptor antagonists,[17] the NO-precursor L-arginine,[16] the NO-donor SIN-1 (unpublished data), and free radical scavengers[31] effectively reduced reperfusion injury. Taking into account that in some of the studies ischemic time[30,31] or assessment protocol[30] 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[16,17] 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[32] 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[33] 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 myoblasts and endothelial cells in the setting of peroxynitrite injury.[6] 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.[34,35] PARP regulates expression of variety of genes including inducible NO synthetase,[36] collagenase,[37] and ICAM-1.[38] 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 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.

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