Journal of the American College of Cardiology
© 2002 by the American College of Cardiology Foundation
Published by Elsevier Science Inc.
Vol. 40, No. 5, 2002
ISSN 0735-1097/02/$22.00
PII S0735-1097(02)02062-4

Pharmacologic Inhibition of Poly(Adenosine Diphosphate-Ribose) Polymerase May Represent a Novel Therapeutic Approach in Chronic Heart Failure

Pál Pacher, MD, PHD,* Lucas Liaudet, MD,†§ Jon G. Mabley, PHD,* Katalin Komjáti, MD, PHD,*‡ Csaba Szabó, MD, PHD*†

Beverly, Massachusetts; Newark, New Jersey; Budapest, Hungary; and Lausanne, Switzerland

OBJECTIVES We investigated the effects of a novel ultrapotent poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitor, PJ34, on cardiac and endothelial dysfunction in a rat model of chronic heart failure (CHF).

BACKGROUND Overactivation of the nuclear enzyme PARP importantly contributes to the development of cell dysfunction and tissue injury in various pathophysiologic conditions associated with oxidative stress, including myocardial reperfusion injury, heart transplantation, stroke, shock, and diabetes.

METHODS Chronic heart failure was induced in Wistar rats by chronic ligation of the left anterior descending coronary artery. Left ventricular (LV) function and ex vivo vascular contractility and relaxation were measured 10 weeks after the surgery. Nitrotyrosine (NT) formation and PARP activation were detected by immunohistochemistry.

RESULTS Chronic heart failure induced increased NT formation and PARP activation in the myocardium and intramural vasculature, depressed LV performance, and impaired vascular relaxation of aortic rings. PJ34 significantly decreased myocardial PARP activation but not NT formation, and improved both cardiac dysfunction and vascular relaxation.

CONCLUSIONS Poly(ADP-ribose) polymerase inhibition represents a novel approach for the experimental treatment of CHF. (J Am Coll Cardiol 2002;40:1006–16) © 2002 by the American College of Cardiology Foundation

Poly(adenosine diphosphate-ribose) polymerase (PARP) is a nuclear enzyme with multiple regulatory functions. The PARP cleaves nicotinamide adenine dinucleotide (NAD^+) to nicotinamide and ADP-ribose to form long branches (ADP-ribose) polymers on glutamic acid residues of a number of target proteins including histones and PARP (automodification domain) itself. Poly(ADP-ribosyl)ation is involved in the regulation of many cellular processes such as deoxyribonucleic acid (DNA) repair, gene transcription, cell cycle progression, cell death, chromatin function, and genomic stability. When PARP is overactivated by oxygen- and nitrogen-derived radical-induced DNA single-strand breaks, it produces extended chains of ADP-ribose on nuclear proteins and results in substantial depletion of intracellular NAD^+. As NADH functions as an electron carrier in the mitochondrial respiratory chain, NAD^+ depletion rapidly leads to falling intracellular adenosine triphosphate levels, eventually leading to cellular dysfunction and death (1) (Fig. 1). Overactivation of PARP represents an important novel mechanism of cell dysfunction in various pathophysiologic conditions associated with increased oxidative stress, including myocardial reperfusion injury (2,3), heart transplantation (4), circulatory shock (5,6), stroke (7), and diabetes mellitus and its complications (8–11). Oxidative stress plays a pathogenetic role in chronic heart failure (CHF) (12–17). We investigated whether pharmacologic inhibition of PARP affects cardiac and vascular function in a rat model of CHF.

From the *Inotek Pharmaceuticals Corporation, Beverly, Massachusetts; †Department of Surgery, New Jersey Medical School, University of Medicine and Dentistry New Jersey, Newark, New Jersey; ‡Institute of Human Physiology and Experimental Research, Semmelweis University, Budapest, Hungary; and the §Critical Care Division, Department of Internal Medicine, University Hospital, Lausanne, Switzerland. Dr. Pacher is on leave from the Institute of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary. This work was supported by a grant from the National Institutes of Health (R01 HL 59266) to Dr. Szabó.

Manuscript received February 7, 2002; revised manuscript received May 15, 2002, accepted May 23, 2002.

MATERIALS AND METHODS

The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health, and was performed with approval of the local Animal Care and Use Committee.

Surgical preparation. Male Wistar rats (Charles River) (97 animals) weighing 300 to 330 g were anesthetized with intraperitoneal thiopentone sodium (60 mg/kg). The animals were intubated under direct vision with polyethylene tubing and placed on artificial ventilation (75 strokes/min, tidal volume = 8 ml/kg, inspired fraction of oxygen = 0.5) using a Harvard model 683 respirator (Harvard, Holliston, Massachusetts). A positive end-expiratory pressure of 5 cm H_2O was applied to minimize atelectasis. The animals were placed on controlled heating pads, and core temperature measured via rectal probe was maintained at 37°C. The animals were given an analgesic (buprenorphine 0.25 mg/kg subcutaneously) before surgery and an identical dose 6 h later.

Myocardial infarction model. Coronary artery ligation was performed in 81 animals, as previously described

Abbreviations and Acronyms

CHF	= chronic heart failure
DNA	= deoxyribonucleic acid
+dP/dt	= systolic pressure increment
−dP/dt	= diastolic pressure decrement
iNOS	= inducible isoform of nitric oxide synthase
LAD	= left anterior descending coronary artery
LV	= left ventricle/ventricular
LVSP	= left ventricular systolic pressure
LVEDP	= left ventricular end-diastolic pressure
NAD^+	= nicotinamide adenine dinucleotide
3-NT	= 3-nitrotyrosine
PARP	= poly(adenosine diphosphate-ribose) polymerase
PBS	= phosphate buffered saline
SNK	= Student-Newman-Keuls

(18,19). Following a left thoracotomy at the fourth intercostal space under aseptic conditions, the pericardium was opened and the heart briefly exteriorized. The left anterior descending (LAD) coronary artery was then permanently ligated by an intramural 5.0 silk suture, inserted from below the tip of the left atrial appendage up to the pulmonary conus. The chest wall was closed in layers using 4.0 silk suture. Animals were rapidly weaned from the ventilator and placed in cages with free access to food and water. A group of 16 rats had the same surgical procedure, except that the LAD was not occluded (sham group).

Treatment protocol. Twenty-four hours after the coronary occlusion, the surviving animals were randomly assigned to receive a daily intraperitoneal injection of an ultrapotent water-soluble PARP inhibitor, PJ34 (9) (20 mg/kg dissolved in 0.5 ml isotonic saline; PJ34 group, n = 16), or isotonic saline (0.5 ml; control group, n = 15). The selected treatment regimen is effective in blocking vascular and cardiac PARP activation, as confirmed by direct measurement of poly(ADP-ribose) formation (9,11,20). The start of the PARP inhibitor treatment was delayed by 24 h to avoid suppression by PARP inhibition of infarct size (18–21).

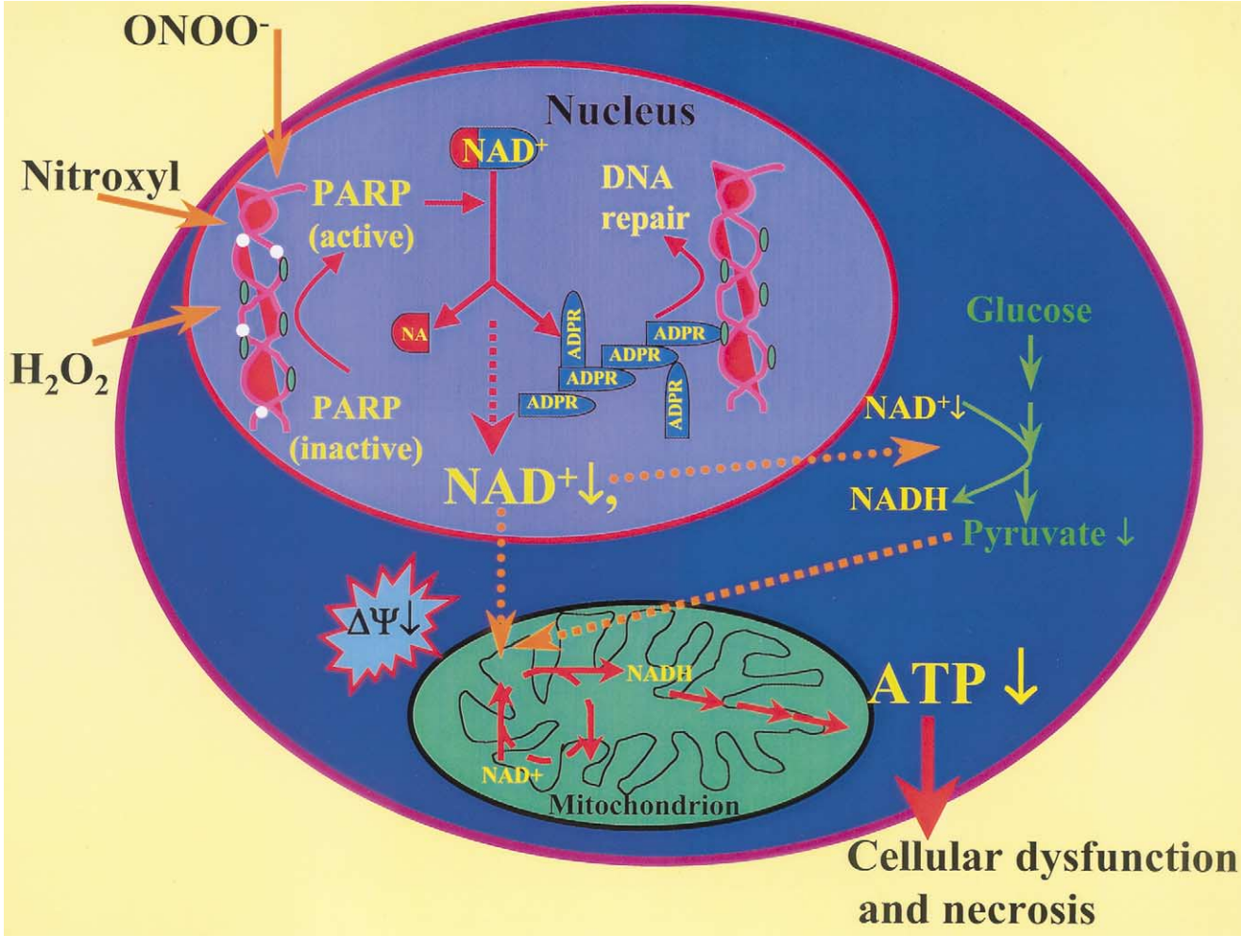

Figure 1. Proposed role of poly(adenosine diphosphate-ribose) polymerase (PARP) in oxidant-induced cellular dysfunction and necrosis. Oxygen and nitrogen-derived radicals cause stand breaks in deoxyribonucleic acid (DNA), activating PARP. Activation of PARP initiates an energy-consuming cycle by transferring adenosine diphosphate-ribose units from nicotinamide adenine dinucleotide (NAD^+) to nuclear proteins resulting in rapid depletion of the intracellular NAD^+ and adenosine triphosphate (ATP) pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death. ADPR = adenosine diphosphate-ribose; NADH = reduced nicotinamide adenine dinucleotide; $ONOO^-$ = peroxynitrite.

JACC Vol. 40, No. 5, 2002
September 4, 2002:1006–16

The sham rats were also given a daily intraperitoneal injection of isotonic saline (0.5 ml; n = 8) or PJ34 (20 mg/kg; n = 8). Treatments continued for 10 weeks.

Measurements. LEFT VENTRICULAR (LV) FUNCTION. At the end of the 10 weeks of observation, LV function was determined as described (11,18). Animals were anesthetized with thiopentone sodium (60 mg/kg intraperitoneally) and tracheotomized to facilitate breathing. Animals were placed on controlled heating pads, and core temperature as measured via a rectal probe was maintained at 37°C. A microtip catheter transducer (SPR-524, Millar Instruments, Houston, Texas) was inserted into the right carotid artery and advanced into the LV under pressure control. After stabilization for 20 min, the pressure signal was continuously recorded using a MacLab A/D converter (AD Instruments, Mountain View, California) and stored and displayed on an Apple Macintosh computer. The heart rate and left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) were measured and the maximal slopes of systolic pressure increment (+dP/dt) and diastolic pressure decrement (−dP/dt), indexes of contractility and relaxation, were calculated. After these measurements, the catheter was pulled back into the aorta to measure of arterial blood pressure.

INFARCT SIZE AND CARDIAC REMODELING. Following the hemodynamic measurements, animals were killed by exsanguination. The heart was removed and the different cavities (left and right ventricles, left and right atria) were dissected and weighed. The weight of each cavity was then normalized to the length of the right tibia. The infarcted (scar) area of the LV was then carefully dissected from the noninfarcted LV, and infarct size was evaluated by computing the ratio of scar tissue weight to the total LV weight. The lungs were also removed and weighed, and wet lung weights were normalized to tibia length and were used as an index of extravascular water lung accumulation.

After these measurements, noninfarcted LVs were fixed in 10% formalin, sliced, and embedded in paraffin for immunohistochemical detection of 3-nitrotyrosine (NT) and poly(ADP-ribose) formation.

IMMUNOHISTOCHEMICAL DETECTION OF NT. Paraffin sections (3 μm) were deparaffinized in xylene and rehydrated in decreasing concentrations (100%, 95%, and 75%) of ethanol followed by a 10-min incubation in phosphate buffered saline (pH 7.4). Sections were treated with 0.3% hydrogen peroxide for 15 min to block endogenous peroxidase activity and then rinsed briefly in PBS. Nonspecific binding was blocked by incubating the slides for 1 h in 0.25% Triton/PBS containing 2% horse serum. Mouse monoclonal anti-nitrotyrosine antibody (Upstate Biotechnology, Lake Placid, New York) and isotype-matched control antibody were applied in a dilution of 1:200 for 1 h at room temperature. After extensive washing (3 × 10 min) with 0.25% Triton/PBS, immunoreactivity was detected with a biotinylated horse antimouse secondary antibody and avidin-biotin-peroxidase complex, both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, California). Color was developed using the Ni-DAB substrate kit (Vector Laboratories). Sections were then counterstained with nuclear fast red, dehydrated, and mounted in Permount. Photomicrographs were taken with a Zeiss Axiolab microscope equipped with a Fuji HC-300C digital camera.

Extent of immunoreactivity in cells was determined by measuring the optical density of NT signal in the myocytes. The background intensity was subtracted from the images and an average optical density of myocytes was determined for each image as a measure of average staining intensity as described (22). A total of 18 to 26 images were examined in each condition from four to five hearts.

IMMUNOHISTOCHEMICAL DETECTION OF POLY(ADP-RIBOSE). Poly(ADP-ribose) was detected as described (11) with minor modifications, as follows. Mouse monoclonal anti-poly(ADP-ribose) antibody (Alexis, San Diego, California) and isotype-matched control antibody were applied in a dilution of 1:600 for 1 h at room temperature. After extensive washing (3 × 10 min) with 0.25% Triton/PBS, immunoreactivity was detected with a biotinylated horse antimouse secondary antibody and avidin-biotin-peroxidase complex, both supplied in the Vector Elite kit (Vector Laboratories). Color was developed using the Ni-DAB substrate kit (Vector Laboratories). Sections were then counterstained with nuclear fast red, dehydrated, and mounted in Permount. Photomicrographs were taken with a Zeiss Axiolab (Charlotte, Vermont) microscope equipped with a Fuji HC-300C digital camera. All histologic and immunohistochemical samples were coded and examined and graded by an investigator in a blinded fashion.

Quantification of the intensity of the poly(ADP-ribose) staining was performed as previously published (11). The percentage of PARP-positive nuclei of cardiomyocytes was obtained by conventional microscopy. A total of 2,532 to 3,452 nuclei profiles were examined in each condition, in at least six different animals in each group. The results are expressed as the percent of PARP-positive nuclei of myocytes relative to the number of total nuclei counted.

VASCULAR FUNCTION IN ISOLATED AORTIC RINGS. Thoracic aortae were removed and cleared from periadventitial fat and cut into 3 to 4 mm width rings using an operation microscope, then mounted in organ baths filled with warmed (37°C) and oxygenated (95% O_2, 5% CO_2) Krebs' solution ($CaCl_2$ 1.6 mM; $MgSO_4$ 1.17 mM; EDTA 0.026 mM; NaCl 130 mM; $NaHCO_3$ 14.9 mM; KCl 4.7 mM; KH_2PO_4 1.18 mM; glucose 11 mM). Isometric tension was measured with isometric transducers (Kent Scientific Corporation, Litchfield, Connecticut), digitized using a MacLab A/D converter, and stored and displayed on a Macintosh computer. A tension of 1.5 g was applied and the rings were equilibrated for 60 min, followed by measurements of the concentration-dependent contraction to epinephrine (10^{-10} to 3×10^{-5} M), and in rings precontracted with epinephrine (10^{-6} M), relaxation to acetylcholine (10^{-9} to 3×10^{-4} M), and sodium nitroprusside (10^{-12} to 10^{-5} M).

Reagents. All reagents were obtained from Sigma/Aldrich (St. Louis, Missouri), unless indicated otherwise. The potent, novel, water-soluble phenanthridinone derivative PARP inhibitor, PJ34—the hydrochloride salt of N-(-oxo-5,6-dihydro-phenanthridin-2-yl)-N,N-dimethylacetamide—was synthesized as described (9). In cell-free PARP assay, using NAD^+ and purified PARP-1 enzyme, PJ34 inhibited PARP activity in a dose-dependent manner, with an EC_{50} of 20 nM. The EC_{50} of the prototypical PARP inhibitor 3-aminobenzamide was 200 μM. Peroxynitrite- and hydrogen peroxide-induced oxidation of dihydrorhodamine-123 were unaffected by PJ34 in the concentration range of 1 μM to 10 mM, indicating that the compound does not act as an antioxidant. The details of the synthesis and pharmacologic characterization of PJ34 were published previously (9).

Statistical analysis. All the data are represented as means and 95% confidence intervals. Data of hemodynamic measurements, infarct size, weight of different cavities, and lung were analyzed with one-way analysis of variance, followed by Student-Newman-Keuls (SNK) multiple comparisons post-hoc analysis. Hemodynamic variables represented, for each animal, the average of four values taken over a 10-min period following stabilization. For the vascular rings studies, dose-response curves were analyzed with analysis of variance for repeated measurements, using a model including the groups as between-subjects factor and concentrations as within-subjects factor. When the F value was significant at the 5% level, the different groups were compared at each concentration level of acetylcholine, sodium nitroprusside, or epinephrine using SNK multiple comparisons post-hoc analysis and Student *t* test. Probability values of $p < 0.05$ were considered significant. For vascular ring studies in most of the animals we used two aortic rings from one rat to avoid the potential error arising from regional heterogeneity of vascular responses. However, only one mean response from each animal was used for final calculation. For all the other measurements we also used only one observation per rat.

RESULTS

Mortality. All the sham animals survived the 10 weeks of the study. Among the 81 rats exposed to permanent ligation of the LAD, 50 animals died during the first 24 h (sudden death attributed to ventricular fibrillation) before the initiation of therapy. In the 31 remaining animals, 3 PJ34-treated and 2 saline-treated rats died during the 10 weeks of observation. At the end of the observation period, the number of surviving animals was thus 13 rats in the PJ34 group and 13 rats in the control groups. All sham animals survived.

Cardiac function. EFFECT OF PJ34 ON THE CARDIAC DYSFUNCTION. Chronic heart failure was characterized by significantly increased LVEDP and decreased LVSP, mean blood pressure, +dP/dt and −dP/dt (Fig. 2). The PJ34 significantly attenuated the CHF-induced suppression of systolic +dP/dt and diastolic −dP/dt and an increase in LVEDP (Fig. 2). The PJ34 significantly improved decreased mean blood pressure in CHF and also tended to increase LVSP (Fig. 2). The PARP inhibitor exerted no significant effects on hemodynamic parameters in sham rats (Fig. 2). The heart rates were not significantly different in all groups studied (Fig. 2).

INFARCT SIZE AND CARDIAC REMODELING. The infarct size in two groups was 28 ± 5% (CHF) and 29 ± 4% (CHF + PJ34), respectively. We cannot rule out the possibility that the method we used to determine the infarct size led to an underestimation of the actual size of the infarct, especially when considering the high mortality rate of our model, reflecting extensive infarction in the dying animals as well as the marked ventricular dysfunction noted in the surviving animals.

Chronic heart failure induced a significant increase in the weights of the LV and left and right atria (Fig. 3). The right ventricular weight also tended to increase. Chronic heart failure was also characterized by increased lung weights, indicative of marked pulmonary edema secondary to cardiac decompensation (Fig. 3). Treatment with PJ34 significantly reduced the CHF-induced increase in LV, left and right atrial, and lung weights (Fig. 3).

EVIDENCE FOR INCREASED NT FORMATION AND PARP ACTIVATION IN CHF IN THE HEARTS IN VIVO. Figure 4 shows widespread increased cardiac NT formation in failing ventricles and intramural vasculature (Fig. 4b), which was not affected by PARP inhibitor treatment (Fig. 4c). The average image NT staining intensity was 14 ± 4 a.u. ($n = 4$) in sham animals, which was significantly ($p < 0.01$; $n = 5$) increased by ~80% and ~64% to 25 ± 3 a.u. and 23 ± 3 a.u. in the CHF and CHF + PJ34 groups, respectively.

As shown in Figure 5, a marked degree of PARP activation was observed in the hearts isolated from rats with CHF (Fig. 5b). The staining was mainly localized in the nuclei of cardiac myocytes and endothelial cells. The PARP activation was attenuated by treatment with PJ34 (Fig. 5c). The percentage of PARP-positive nuclei of myocytes was 16 ± 3 ($n = 6$) in sham animals. Chronic heart failure increased the fraction of PARP-positive nuclei to 42 ± 5 ($p < 0.01$; $n = 8$). Treatment with PJ34 (for 10 weeks) markedly decreased the percentage of PARP-positive nuclei to 23 ± 2 ($p < 0.01$; $n = 6$) in rats with CHF.

PARP INHIBITION IMPROVES CHF-INDUCED ENDOTHELIAL DYSFUNCTION IN RATS. In agreement with previous reports (23–25), ex vivo experiments demonstrated the loss of endothelial function in rats with CHF (Fig. 6). Inhibition of PARP restored normal vascular function in rats with CHF (Fig. 6). Contractile and endothelium-independent relaxant responses to sodium nitroprusside were unchanged (Fig. 6).

DISCUSSION

A multitude of new pharmacologic approaches have been introduced during the past decade for experimental therapy

JACC Vol. 40, No. 5, 2002
September 4, 2002:1006–16

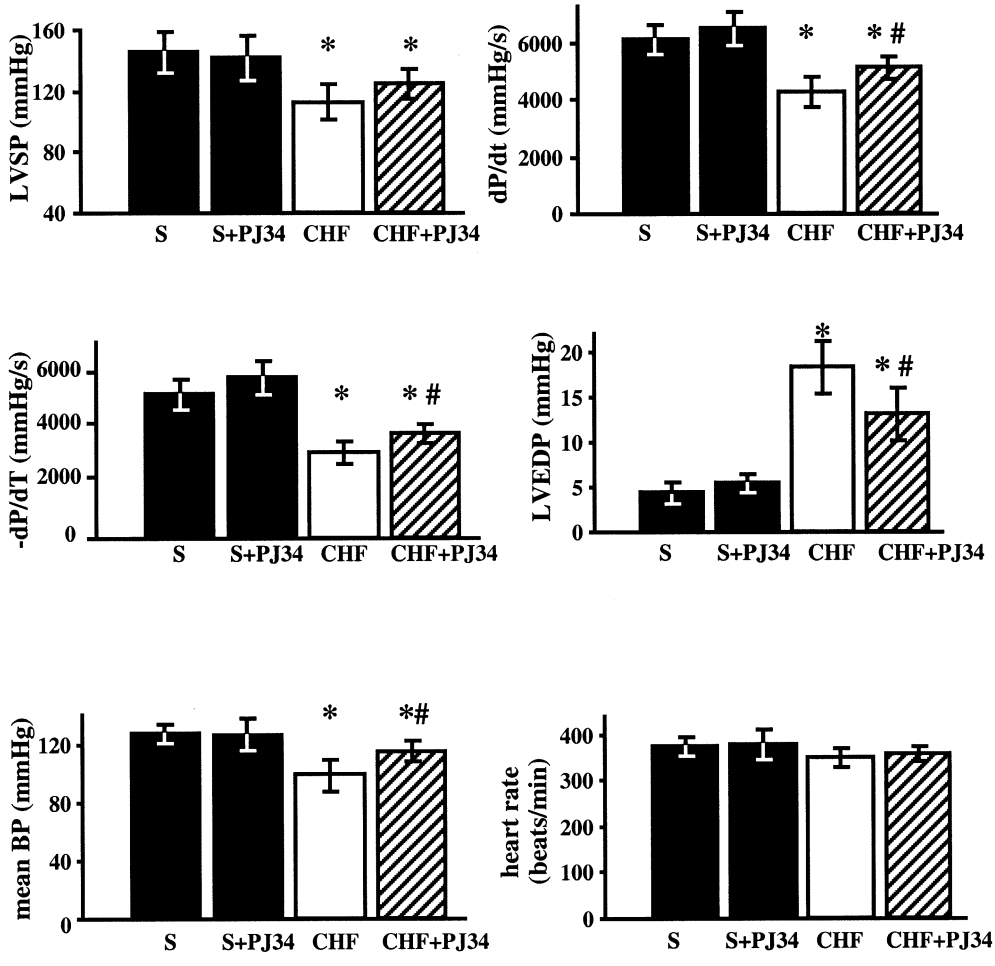

Figure 2. Improvement of cardiac dysfunction by pharmacologic inhibition of poly(adenosine diphosphate-ribose) polymerase 10 weeks after permanent ligation of left anterior descending coronary artery in a rat model of chronic heart failure (CHF). Effect of CHF and PJ34 on left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular −dP/dt in rats. Results are mean ± 95% confidence interval of 8 to 10 experiments in each group. *$p < 0.05$ vs. S; #$p < 0.05$ vs. CHF. BP = blood pressure; CHF + PJ34 = chronic heart failure treated with PJ34 (for 10 weeks); S = sham; S + PJ34 = sham treated with PJ34 (for 10 weeks).

of CHF. Although many of these approaches have been successful in animal models and clinical trials, morbidity and mortality from CHF remain high, and research into pathomechanisms of disease and new disease-modifying pharmacologic targets continues.

The present study demonstrates that CHF induced by chronic ligation of the LAD in rats was associated with a marked depression of LV function, impaired relaxation of aortic rings to acetylcholine, and decreased blood pressure without change in the heart rate. These results are consistent with earlier reports using similar rat models (14,26,27). The reduction in blood pressure is most likely related to reduction in cardiac output while systemic resistance is generally increased or unchanged (28). The results presented here document for the first time that in an experimental model of CHF, the impaired cardiac and vascular function is associated with an activation of PARP in the myocardium and vasculature. Furthermore, we now show that the cardiac and endothelial dysfunction is improved by pharmacologic inhibition of PARP. Because we did not measure cardiac output and calculate systemic vascular resistance, we cannot completely rule out the possibility that some of the beneficial effects of PJ34 on heart function were related to its favorable influence on the peripheral circulation. However, on the basis of the results of previous investigations and our data presented in the current study, it appears that PJ34 improved cardiac function by direct influence on the heart itself. Indeed, in several studies performed in isolated working hearts exposed to ischemia and reperfusion, it has been shown that suppression of PARP activity, either by pharmacologic (2,29,30) or by genetic deletion of the PARP gene (31), produced a significant improvement of cardiac function. In a study by Pieper et al., PARP activation was directly demonstrated in isolated hearts from wild-type mice following ischemia and reperfusion (32). In contrast, PARP activation was not detectable in hearts from PARP-deficient animals, and the lack of PARP was associated with significant improvement of cardiac contractility. These observations support a) that PARP activation produces direct detrimental influence on

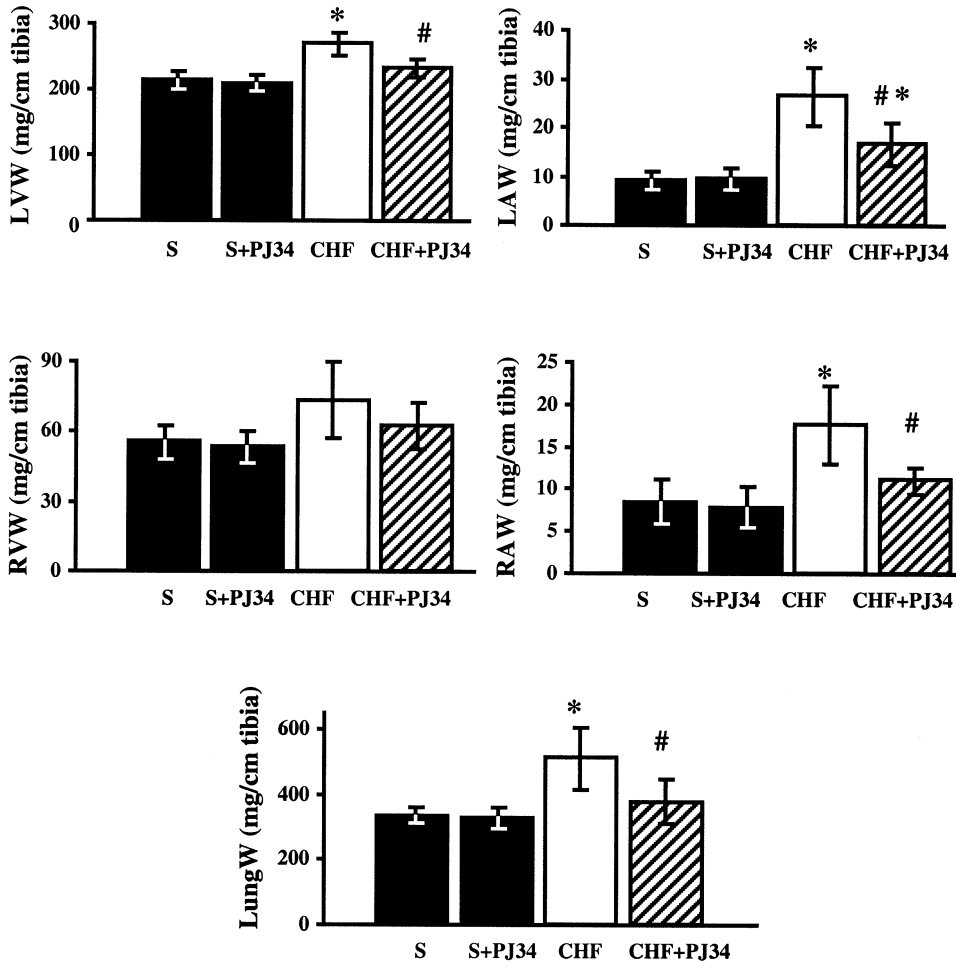

Figure 3. Effect of pharmacologic inhibition of poly(adenosine diphosphate-ribose) polymerase on cardiac remodeling and lung weight in rat model of chronic heart failure (CHF) induced by permanent ligation of left anterior descending (LAD) coronary artery. Left ventricular (LVW), right ventricular (RVW), left atrial (LAW), right atrial (RAW), and lung (LungW) weights in sham (S), PJ34-treated (for 10 weeks) sham (S + PJ34), CHF and PJ34-treated (for 10 weeks) CHF (CHF + PJ34) rats. Results are mean ± 95% confidence interval of 8 to 10 experiments in each group. The weight of each cavity and lung was normalized to the length of the right tibia. *$p < 0.05$ vs. S; #$p < 0.05$ vs. CHF.

cardiac function, and b) that PARP suppression can exert direct benefit on cardiac function, independent from any peripheral vascular effects.

Experimental and clinical studies have suggested an increased production of reactive oxygen species (superoxide, hydrogen peroxide, hydroxyl radical) in the failing myocardium (12–17,25). The expression and activity of inducible isoform of nitric oxide synthase (iNOS) is also increased in the myocardium of patients with CHF (33,34). Importantly, a recent study has suggested correlation between chronic overexpression of iNOS and peroxynitrite generation with cardiac enlargement, conduction defects, sudden cardiac death and, less commonly, heart failure in mice (35). Myocardial iNOS is also activated in rats with volume-overload HF, and increased iNOS activity contributes to depressed myocardial contractility and beta-adrenergic hyporesponsiveness (36). The combination of NO and superoxide yields peroxynitrite (37), which is able to trigger DNA single-strand breakage and activation of PARP (1,5). Peroxynitrite generation has been demonstrated in various models of CHF, and this species has been shown to impair cardiac function via multiple mechanisms (15,38). In the current study, similarly to that of Mihn et al. (15), we also demonstrate widespread increased NT formation in failing ventricles. Importantly, the increased NT formation in CHF is not reduced by PARP inhibitor treatment, which is consistent with our proposed scheme, where PARP activation lies downstream from the generation of oxidants.

A recently characterized mechanism of myocardial dysfunction involves the activation of the nuclear enzyme PARP by DNA single-strand breaks generated in response to increased oxidative and nitrosative stress. Evidence for the importance of this pathway has been demonstrated in hearts subjected to regional or global ischemia and reperfusion (2,3,18–21,29–32). The mode of PARP inhibitors' cardioprotective action involves a conservation of myocardial energetics, as well as a prevention of the upregulation of various proinflammatory pathways (cytokines, adhesion receptors, mononuclear cell infiltration) triggered by ischemia and reperfusion (3,21,29). Similar to acute myocardial

JACC Vol. 40, No. 5, 2002
September 4, 2002:1006–16

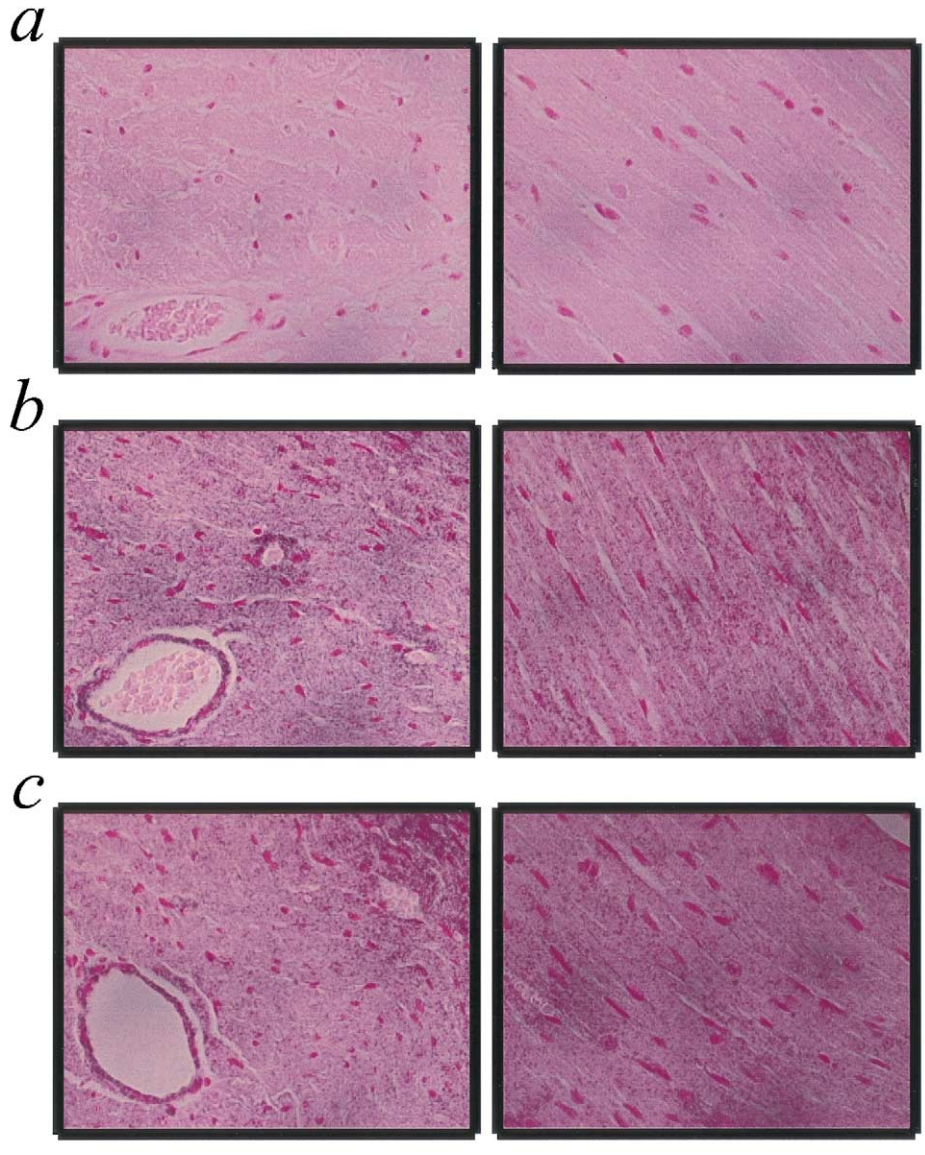

Figure 4. Evidence for increased nitrotyrosine (NT) formation in hearts from rats with chronic heart failure (CHF). Immunohistochemical staining for NT, an indicator of peroxynitrite formation, in sham **(a)**, CHF **(b)**, and PJ34-treated CHF **(c)** rat hearts. **Panel b** shows widespread NT formation in myocytes and intramural vasculature in rats with CHF. Treatment with PJ34 for 10 weeks **(c)** did not reduce NT formation in rat hearts with CHF. Similar immunohistochemical profiles were seen in n = 4 to 5 hearts per group.

ischemia and reperfusion, both oxidant-mediated myocardial injury and upregulation of various proinflammatory processes play important roles in CHF. It is conceivable that PARP inhibition exerts beneficial effects in the current model by affecting both pathways of injury, and also by suppressing positive feedback cycles they initiate.

We previously showed that massive PARP activation contributes to necrotic cell death in the region of acute myocardial infarct in rats and pigs (18,20). In the current study we have delayed the start of PARP inhibitors' administration to 24 h to prevent an effect on the primary process of infarction, and to focus on the delayed pathophysiologic processes in the noninfarcted myocardial tissue.

Chronic heart failure is characterized by impaired NO-mediated vasodilation (39–41), which may contribute to increased peripheral vascular resistance. The pathogenesis of endothelial dysfunction associated with CHF appears to involve enhanced oxidative stress from various local sources (27,41), including iNOS, which in CHF paradoxically appears to produce superoxide rather than NO (42). Reactive oxidant species, including the cytotoxic oxidant peroxynitrite, are generated in various forms of vascular injury (including circulatory shock, diabetes, and reperfusion injury), and are responsible for the impairment of endothelium-dependent relaxations, in part via activation of PARP in the endothelium (5,9–11). Importantly, a recent report has demonstrated marked decreased NO availability and enhanced superoxide and peroxynitrite production in vasculature of normotensive and hypertensive rats with CHF induced by occlusion of the left coronary artery (43).

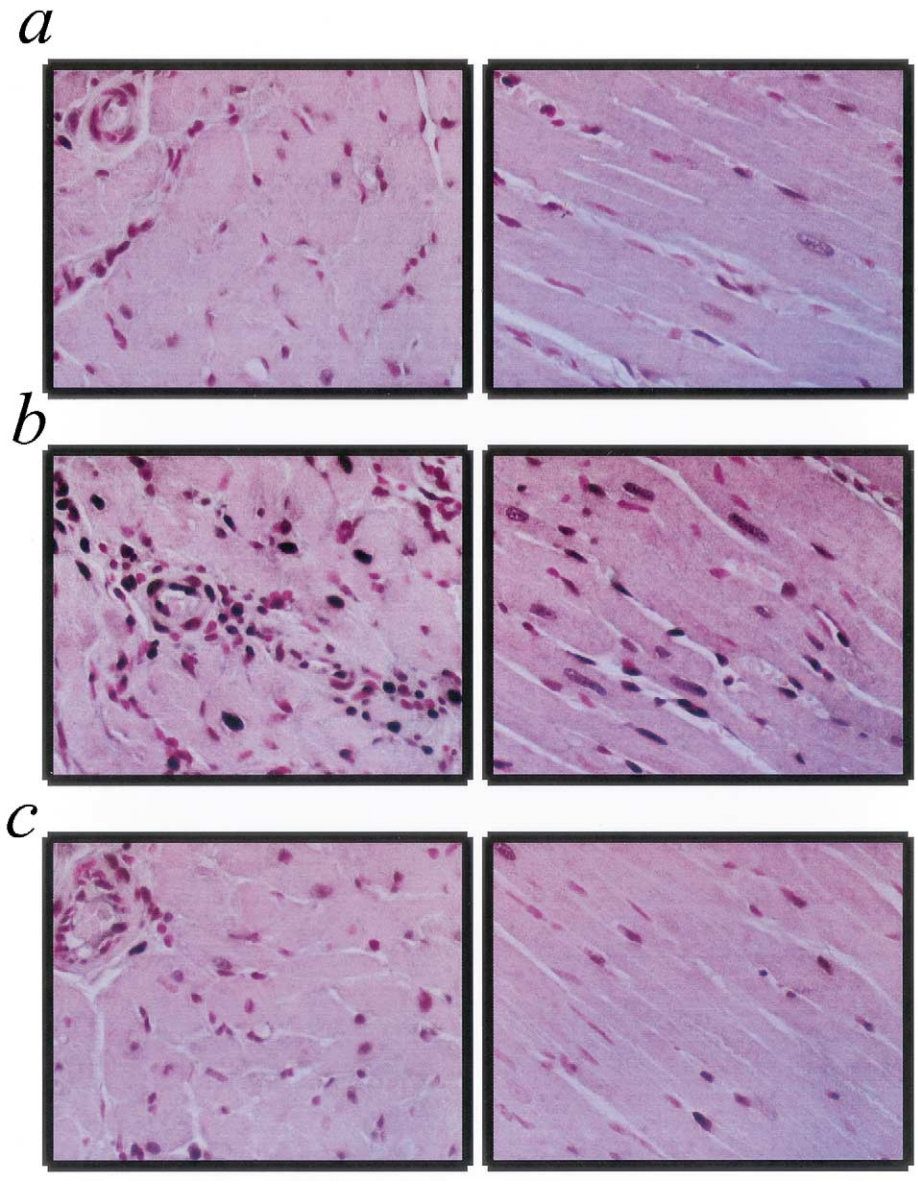

Figure 5. Evidence for poly(adenosine diphosphate-ribose) polymerase (PARP) activation in hearts from rats with chronic heart failure (HF). Immunohistochemical staining for poly(ADP-ribose) formation, an indicator of PARP activation, in sham **(a)**, CHF **(b)**, and PJ34-treated CHF **(c)** rat hearts. **Panel b** shows poly(ADP-ribose) formation localized in the nuclei of myocytes and endothelial cells in rats with CHF. Treatment with PJ34 for 10 weeks **(c)** markedly reduced PARP activation in rats hearts with CHF. Similar immunohistochemical profiles were seen in six to eight hearts per group.

Consistent with these results, we also found increased NT formation in intramural vasculature (Fig. 4) in failing ventricles.

Our results obtained with PJ34 clearly demonstrate that the impairment of the cardiac and endothelial function in CHF—at least in the current experimental model—are dependent on PARP activity. We propose that following the myocardial infarction, the compensatory increased sympathetic activity, the activated renin-angiotensin system, and the increased workload of the noninfarcted regions lead to myocardial hypertrophy, myocardial ischemia/functional hypoxia, increased oxidative and nitrosative stress, and PARP activation, which in turn results in rapid depletion of the intracellular NAD^+ and ATP pools, leading to cardiac and vascular dysfunction. It is conceivable that the endothelial PARP pathway and the cardiomyopathy in CHF are interrelated: an impairment of the endothelial function may lead to global or regional myocardial ischemia, which may secondarily impair cardiac performance.

As with any compound, we cannot fully exclude the possibility that PJ34 may also act at yet undetermined pharmacologic sites other than PARP in the heart. However, PJ34 is one of the most potent, selective, and effective bioavailable PARP inhibitors discovered to date (9), and in contrast to some other pharmacologic inhibitors of PARP (such as nicotinamide and 3-AB), it lacks antioxidant effects (9) (Fig. 5). We have previously shown that the dose regimen of PJ34 used in the current study effectively inhibits

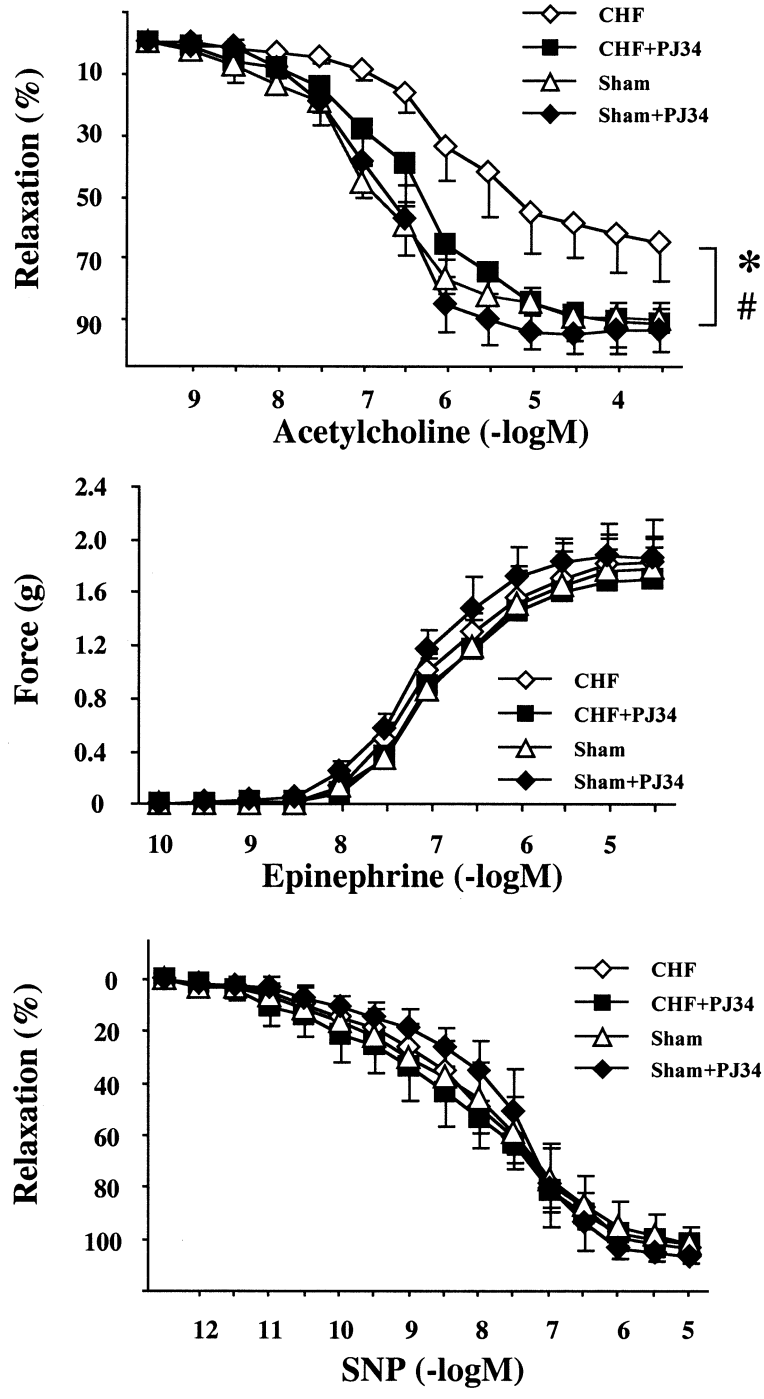

Figure 6. Reversal of chronic heart failure (CHF)-induced endothelial dysfunction by pharmacologic inhibition of poly(ADP-ribose) polymerase (PARP) in rats: epinephrine-induced contractions **(middle)**, acetylcholine-induced endothelium-dependent relaxation **(upper)**, and sodium nitroprusside (SNP)-induced endothelium-independent relaxations **(lower)**. Each point of the curve represents mean ± 95% confidence interval of 7 to 10 experiments in vascular rings. *$p < 0.05$ vs. S; #$p < 0.05$ vs. CHF. S = sham.

PARP activation in the myocardium (4,11,20) under various pathophysiologic conditions, and the current study also confirmed this finding. Because of the similar protection seen with PARP-deficient animals and PJ34 in various animal models of cardiovascular disorders and immunohistochemical results included in the current and other studies, it is probable that PJ34 works via selective inhibition of PARP activity (2,4,9–11,20,31,32). This is also supported by our recent results demonstrating that in a mouse model of doxorubicin-induced acute heart failure, both genetic deletion of PARP and pharmacologic inhibition with PJ34 were associated with better preservation of myocardial performance (44).

In conclusion, our study provides evidence that the PARP activation importantly contributes to the pathogenesis of cardiovascular dysfunction in an experimental model of

CHF. Inhibition of PARP may represent a novel approach for the experimental therapy of CHF.

Reprint requests and correspondence: Dr. Csaba Szabó, Inotek Corporation, Suite 419E, 100 Cummings Center, Beverly, Massachusetts 01915. E-mail: szabocsaba@aol.com.

REFERENCES

1. Szabó C, editor. Cell Death: The Role of PARP. Boca Raton, FL: CRC Press, 2000.
2. Thiemermann C, Bowes J, Myint FP, Vane JR. Inhibition of the activity of poly (ADP ribose) synthetase reduces ischemia-reperfusion injury in the heart and skeletal muscle. Proc Natl Acad Sci U S A 1997;94:679–83.
3. Zingarelli B, Salzman AL, Szabó C. Genetic disruption of poly(ADP-ribose) synthetase inhibits the expression of P-selectin and intercellular adhesion molecule-1 in myocardial ischemia/reperfusion injury. Circ Res 1998;83:85–94.
4. Szabó G, Bahrle S, Stumpf N, et al. Poly(ADP-ribose) polymerase inhibition reduces reperfusion injury after heart transplantation. Circ Res 2002;90:100–6.
5. Szabó C, Cuzzocrea S, Zingarelli B, O'Connor M, Salzman AL. Endothelial dysfunction in a rat model of endotoxic shock. Importance of the activation of poly(ADP-ribose) synthetase by peroxynitrite. J Clin Invest 1997;100:723–35.
6. Liaudet L, Soriano FG, Szabó È, et al. Protection against hemorrhagic shock in mice genetically deficient in poly(ADP-ribose) polymerase. Proc Natl Acad Sci U S A 2000;97:10203–8.
7. Eliasson MJ, Sampei K, Mandir AS, et al. Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat Med 1997;3:1089–95.
8. Burkart V, Wang ZQ, Radons J, et al. Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozotocin. Nat Med 1999; 5:314–9.
9. Soriano FG, Virág L, Jagtap P, et al. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. Nat Med 2001; 7:108–13.
10. Soriano FG, Pacher P, Mabley J, Liaudet L, Szabó C. Rapid reversal of the diabetic endothelial dysfunction by pharmacological inhibition of poly(ADP-ribose) polymerase. Circ Res 2001;89:684–91.
11. Pacher P, Liaudet L, Soriano FG, Mabley JG, Szabó È, Szabó C. The role of poly(ADP-ribose) polymerase in the development of cardiovascular dysfunction in diabetes mellitus. Diabetes 2002;51:514–21.
12. Ide T, Tsutsui H, Kinugawa S, et al. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. Circ Res 1999;85:357–63.
13. Ekelund UE, Harrison RW, Shokek O, et al. Intravenous allopurinol decreases myocardial oxygen consumption and increases mechanical efficiency in dogs with pacing-induced heart failure. Circ Res 1999; 85:437–45.
14. Bauersachs J, Bouloumie A, Fraccarollo D, Hu K, Busse R, Ertl G. Endothelial dysfunction in chronic myocardial infarction despite increased vascular endothelial nitric oxide synthase and soluble guanylate cyclase expression: role of enhanced vascular superoxide production. Circulation 1999;100:292–8.
15. Mihm MJ, Coyle CM, Schanbacher BL, Weinstein DM, Bauer JA. Peroxynitrite induced nitration and inactivation of myofibrillar creatine kinase in experimental heart failure. Cardiovasc Res 2001;49:798–807.
16. Cappola TP, Kass DA, Nelson GS, et al. Allopurinol improves myocardial efficiency in patients with idiopathic dilated cardiomyopathy. Circulation 2001;104:2407–11.
17. Cesselli D, Jakoniuk I, Barlucchi L, et al. Oxidative stress-mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. Circ Res 2001;89:279–86.
18. Liaudet L, Szabó È, Timashpolsky L, Virag L, Cziraki A, Szabó C. Suppression of poly(ADP-ribose) polymerase activation by 3-aminobenzamide in a rat model of myocardial infarction: long-term morphological and functional consequences. Br J Pharmacol 2001;133: 1424–30.
19. Liaudet L, Yang Z, Al-Affar EB, Szabo C. Myocardial ischemic preconditioning in rodents is dependent on poly(ADP-ribose) synthetase. Mol Med 2001;7:406–17.
20. Faro R, Toyoda Y, McCully JD, et al. Myocardial protection by PJ34, a novel potent poly(ADP-ribose) synthetase inhibitor. Ann Thorac Surg 2002;73:575–81.
21. Zingarelli B, Cuzzocrea S, Zsengeller Z, Salzman AL, Szabó C. Protection against myocardial ischemia and reperfusion injury by 3-aminobenzamide, an inhibitor of poly(ADP-ribose) synthetase. Cardiovasc Res 1997;36:205–15.
22. Pacher P, Hajnoczky G. Propagation of the apoptotic signal by mitochondrial waves. EMBO J 2001;20:4107–21.
23. Teerlink JR, Clozel M, Fischli W, Clozel JP. Temporal evolution of endothelial dysfunction in a rat model of chronic heart failure. J Am Coll Cardiol 1993;22:615–20.
24. Driss AB, Devaux C, Henrion D, et al. Hemodynamic stresses induce endothelial dysfunction and remodeling of pulmonary artery in experimental compensated heart failure. Circulation 2000;101:2764–70.
25. Indik JH, Goldman S, Gaballa MA. Oxidative stress contributes to vascular endothelial dysfunction in heart failure. Am J Physiol 2001; 281:H1767–70.
26. Bauersachs J, Galuppo P, Fraccarollo D, Christ M, Ertl G. Improvement of left ventricular remodeling and function by hydroxymethylglutaryl coenzyme a reductase inhibition with cerivastatin in rats with heart failure after myocardial infarction. Circulation 2001;104:982–5.
27. Francis J, Weiss RM, Wei SG, Johnson AK, Felder RB. Progression of heart failure after myocardial infarction in the rat. Am J Physiol Regul Integr Comp Physiol 2001;281:1734–45.
28. Nagaya N, Uematsu M, Kojima M, et al. Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. Circulation 2001;104: 2034–8.
29. Docherty JC, Kuzio B, Silvester JA, Bowes J, Thiemermann C. An inhibitor of poly(ADP-ribose) synthetase activity reduces contractile dysfunction and preserves high energy phosphate levels during reperfusion of the ischaemic rat heart. Br J Pharmacol 1999;127:1518–24.
30. Szabados E, Literati-Nagy P, Farkas B, Sumegi B. BGP-15, a nicotinic amidoxime derivate protecting heart from ischemia reperfusion injury through modulation of poly(ADP-ribose) polymerase. Biochem Pharmacol 2000;59:937–45.
31. Grupp IL, Jackson TM, Hake P, Grupp G, Szabó C. Protection against hypoxia-reoxygenation in the absence of poly(ADP-ribose) synthetase in isolated working hearts. J Mol Cell Cardiol 1999;31: 297–303.
32. Pieper AA, Walles T, Wei G, et al. Myocardial postischemic injury is reduced by poly(ADP-ribose) polymerase-1 gene disruption. Mol Med 2000;6:271–82.
33. Fukuchi M, Hussain SN, Giaid A. Heterogeneous expression and activity of endothelial and inducible nitric oxide synthases in end-stage human heart failure: their relation to lesion site and beta-adrenergic receptor therapy. Circulation 1998;98:132–9.
34. Vejlstrup NG, Bouloumie A, Boesgaard S, et al. Inducible nitric oxide synthase (iNOS) in the human heart: expression and localization in congestive heart failure. J Mol Cell Cardiol 1998;30:1215–23.
35. Mungrue IN, Gros R, You X, et al. Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. J Clin Invest 2002;109:735–43.
36. Gealekman O, Abassi Z, Rubinstein I, Winaver J, Binah O. Role of myocardial inducible nitric oxide synthase in contractile dysfunction and beta-adrenergic hyporesponsiveness in rats with experimental volume-overload heart failure. Circulation 2002;105:236–43.
37. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. Am J Physiol 1996;271:C1424–37.
38. Ferdinándy P, Danial H, Ambrus I, Rothery RA, Schulz R. Peroxynitrite is a major contributor to cytokine-induced myocardial contractile failure. Circ Res 2000;87:241–7.
39. Drexler H, Hayoz D, Munzel T, et al. Endothelial function in chronic congestive heart failure. Am J Cardiol 1992;69:1596–601.
40. Ramsey MW, Goodfellow J, Jones CJ, Luddington LA, Lewis MJ, Henderson AH. Endothelial control of arterial distensibility is impaired in chronic heart failure. Circulation 1995;92:3212–9.
41. Bauersachs J, Fleming I, Fraccarollo D, Busse R, Ertl G. Prevention of endothelial dysfunction in heart failure by vitamin E: attenuation of

vascular superoxide anion formation and increase in soluble guanylyl cyclase expression. Cardiovasc Res 2001;51:344–50.
42. Miller AA, Megson IL, Gray GA. Inducible nitric oxide synthase-derived superoxide contributes to hyperreactivity in small mesenteric arteries from a rat model of chronic heart failure. Br J Pharmacol 2000;131:29–36.
43. Wiemer G, Itter G, Malinski T, Linz W. Decreased nitric oxide availability in normotensive and hypertensive rats with failing hearts after myocardial infarction. Hypertension 2001;38:1367–71.
44. Pacher P, Liaudet L, Bai P, et al. Activation of poly(ADP-ribose) polymerase contributes to development of doxorubicin-induced heart failure. JPET 2002;300:862–7.