Myocardial Protection by PJ34, a Novel Potent Poly (ADP-Ribose) Synthetase Inhibitor

Renato Faro, PhD, Yoshiya Toyoda, MD, James D. McCully, PhD, Prakash Jagtap, PhD, Eva Szabo, MD, Laszlo Virag, MD, PhD, Cesario Bianchi, MD, PhD, Sidney Levitsky, MD, Csaba Szabo, MD, PhD, and Frank W. Sellke, MD

Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, and Inotek Corporation, Beverly, Massachusetts

Background. **The activation of poly (ADP-ribose) synthetase plays an important role in the pathogenesis leading to myocardial ischemia-reperfusion injury. The aim of this study was to determine if a novel potent inhibitor of poly (ADP-ribose) synthetase, PJ34, provides myocardial protection.**

Methods. **Pigs were subjected to 60 minutes of regional ischemia followed by 180 minutes of reperfusion. Ten mg/kg of PJ34 (PJ34; n = 6) was administrated intravenously (treated group) from 15 to 5 minutes before reperfusion followed by 3 mg/kg/hour of PJ34 from 5 minutes before reperfusion to the end of 180 minutes reperfusion. Control pigs (n = 7) received vehicle only. Arterial and left ventricular pressure and coronary flow were monitored.**

Results. **The PJ34 showed significant reduction on infarct size (37.5% ± 4.5% and 50.5% ± 4.8% of the area at risk) for PJ34 and control pigs groups, respectively, ($p < 0.05$). Significant reduction in postsystolic shortening, as well as improvement on segment shortening, and positive first derivative of pressure over time (+dP/dt) maximum were also observed in PJ34 versus control pigs ($p < 0.05$).**

Conclusions. **Our results suggest that PJ34 provides cardioprotection by decreasing myocardial infarct size and enhancing postischemic regional and global functional recovery.**

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Poly (ADP ribose) synthetase (PARS), also termed poly (ADP ribose) polymerase is an abundant nuclear enzyme of eukaryotic cells that has been shown to be activated in response to DNA injury [1]. Activation of PARS is triggered by oxidant-mediated DNA single-strand breaks and initiates an energy-consuming futile intracellular cycle, leading to the rapid depletion of cellular stores of NAD^+ (substrate of PARS) and adenosine triphosphate, resulting in cell dysfunction, necrosis, and death [2]. Activation of PARS has been also implicated in several pathophysiologic conditions, including ischemia-reperfusion injury [3], inflammation [4], and hemorrhagic shock [5].

The PARS inhibition reduces the velocity of adenosine triphosphate and NAD^+ depletion, improving the survival of several cultured cell types (eg, fibroblasts, endothelium, and vascular smooth muscle) exposed to oxygen-derived free radicals [6, 7], or peroxynitrite [8]. The formation of free radicals contributes to the reperfusion injury in previously ischemic organs, including skeletal muscle and heart [9, 10].

The PARS inhibitors, such as benzamide analogs, nicotinamide, and isoquinoline derivatives, have been used previously in both in vivo and in vitro studies to investigate the role of this nuclear enzyme in various pathophysiologic conditions [2, 11, 12]. The objective of the present study was to characterize the effect of the novel potent phenanthridinone PARS inhibitor (PJ34) [13] in an in vivo model of heart ischemia-reperfusion in the anesthetized pig.

Material and Methods

Animals

Animals were housed individually and provided with laboratory chow and water ad libitum. All experiments were approved by the Beth Israel Deaconess Medical Center Animal Care and Use Committee, and the Harvard Medical Area Standing Committee on Animals (Institutional Animal Care and Use Committee), and conformed to the "Guide for the Care and Use of Laboratory Animals" prepared by the National Institutes of Health (NIH Publication No. 5377–3, 1996).

Surgical Preparation

Pigs of either sex (35 to 45 kg) were sedated with ketamine hydrochloride (20 mg/kg, intramuscularly, Ab-

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Address reprint requests to Dr Sellke, Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, 110 Francis St, No. LMOB Suite 2A, Boston, MA 02215; fsellke@caregroup.harvard.edu.

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bott Laboratories, North Chicago, IL) and anesthetized with sodium pentobarbital (25 mg/kg, intravenously, Abbott Laboratories, North Chicago, IL). General anesthesia was maintained throughout the experiment with sodium pentobarbital. A tracheotomy was performed through a midline cervical incision (36 French Argyle), and ventilation begun with a volume-cycled ventilator: oxygen, 40%; tidal volume, 1000 mL; ventilation rate, 12 breaths per minute; positive end-expiratory pressure, 3 cm H_20; inspiratory to expiratory time ratio, 1:2 (North American Drager, model Narkomed II, Telford, England). The right internal jugular vein was cannulated for intravenous access and injection and the right common carotid artery was cannulated for arterial blood sampling and mean arterial blood pressure (MABP) monitoring (Millar Instruments, Houston, TX). Heparin sodium (Elkins-Sinn, Inc, Cherry Hill, NJ; 5000 IU intravenously) and 1% lidocaine (Elkins-Sinn, Inc, Cherry Hill, NJ; 5 mL intravenously) were given before thoracotomy. Heparin was administered at the same dose every 30 minutes to the end of the experiment. The pericardial sac was exposed through a median sternotomy and was opened to form a pericardial cradle. A catheter-tipped manometer (Millar Instruments, Houston, TX) was introduced through the apex into the left ventricle to record left ventricular pressure. The distal third portion of the left anterior descending artery or its large second diagonal branch was dissected, and a silk thread (0 Silk, Ethicon, Inc, Somerville, NJ) was passed around the left anterior descending artery after the second diagonal. Both ends of the silk tie were threaded through a small vinyl tube to form a snare. The coronary artery was occluded by pulling the snare, which was then secured by clamping the tube with a mosquito clamp. Myocardial ischemia was confirmed visually by regional cyanosis of the myocardial surface [14].

Experimental Protocol

Pigs were randomly divided into two groups. After 60 minutes equilibrium hearts were subjected to 60 minutes regional ischemia followed by 180 minutes reperfusion. Forty-five minutes after the initiation of regional ischemia, control pigs (CT) (n = 6) received a phosphate buffered saline (PBS) infusion through the right internal jugular vein (vehicle control), which was continued throughout the experiment. The PJ34 pigs (PJ34) (n = 6) received an initial high concentration infusion of PJ34 (l0 mg/kg intravenously; Inotek Corp, Beverly, MA) through the right internal jugular vein for 10 minutes, starting at 45 minutes after the initiation of ischemia, followed by a maintenance infusion of PJ34 (3 mg/kg/hour intravenously) to the termination of 180 minutes reperfusion. The PJ34, a novel poly (ADP-ribose) synthetase inhibitor, was synthesized as previously described [13] (Fig 1). Hemodynamic variables were continuously acquired throughout the experiment using a PONE-MAH digital data acquisition system (Gould Instruments, Valley View, OH), with an Acquire Plus processor board, and left ventricular pressure analysis

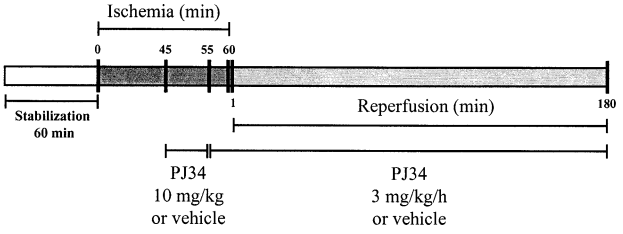

Fig 1. Experimental design. After 60 minute stabilization period the pigs were subject to a coronary artery occlusion, and after 45 minutes of ischemia PJ34 (10 mg/kg) or vehicle was started for 10 minutes. At 55 minutes of ischemia the concentration of PJ34 was reduced to 3 mg/kg/hour and continued throughout the experiment.

software, and a Gould ECG/Biotach (Gould Instruments, Valley View, OH) [14].

Regional Myocardial Function

Regional myocardial function was assessed by sonomicrometry (Sonometrics Digital Ultrasonic Measurement System, Sonometrics Corp, London, CA) using 5 digital piezoelectric ultrasonic probes (2.0 mm) implanted in the subendocardial layer, approximately 10 mm apart within the ischemic area, with two pairs placed parallel to the minor axis of the heart and secured to the epicardium with polypropylene stitches (5-0 Prolene, Ethicon, Somerville, NJ). The probes were left in place until the end of the experiment. Digital data were inspected for correct identification of end-diastolic and end-systolic points using postprocessing software (Sono View, Sonometrics Corp, London, CA) [14]. Measurements were made over at least three cardiac cycles in normal sinus rhythm and then averaged. The ventilator was stopped during data acquisition to eliminate the effects of respiration. End-diastolic segment length was measured at the onset of positive first derivative of pressure over time (+dP/dt), and the end-systolic segment length at peak negative dP/dt (−dP/dt). Regional contractility was assessed by segment shortening (SS). Wall motion abnormalities were assessed as systolic bulging defined as the bulging of the myocardium after the end of diastole. Postsystolic shortening is the shortening after the end of systolic ejection. Time course changes in percent of SS were calculated from the mean ± SEM of 4 to 5 unique horizontal or longitudinal distances, or both, and were expressed as a percent of baseline to minimize variability among individual animals. Time course changes in SS were expressed as a percentage of equilibrium values to minimize variability among individual animals.

Coronary Blood Flow, Blood Gases, and Hematocrit

Coronary blood flow was continually monitored using a transit time ultrasonic flow probe (3 mm RS-Series, Transonic Systems Inc, Ithaca, NY) placed around the left anterior descending coronary artery connected to a T206 flowmeter (Transonic Systems Inc, Ithaca, NY). Blood gases and hematocrit were monitored every 10 to 15 minutes using a Corning 238 pH/blood gas analyzer and a Corning 270 CO-oximeter (Chiron Diagnostics,). Blood

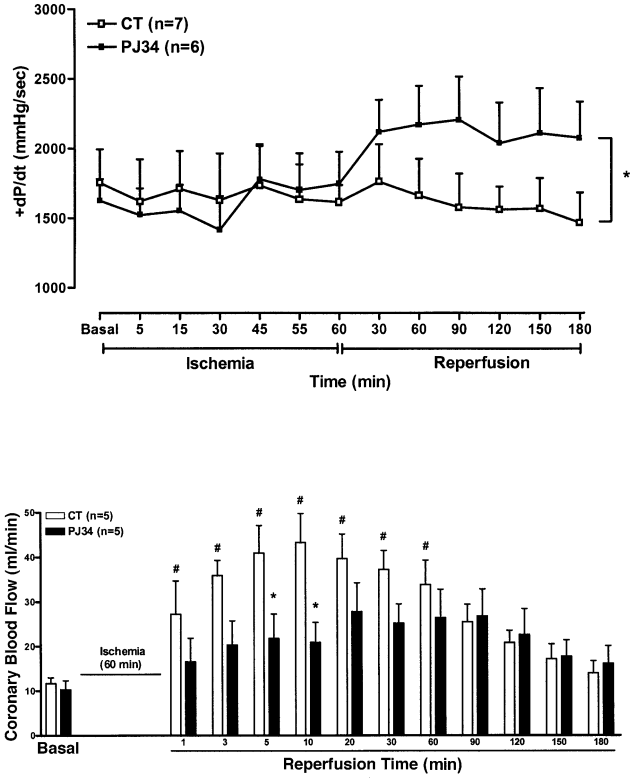

*Fig 2. Upper chart shows the evolution of positive first derivative of pressure over time (+dP/dt) during 60 minutes after coronary artery occlusion followed by 180 minutes reperfusion in control pigs (CT) (□) and PJ34 treated pigs (■) and lower chart shows the effect of ischemia for 60 minutes and reperfusion for 180 minutes in coronary blood flow. The coronary blood flow was increased immediately upon reperfusion. Peaks were observed between 5 and 10 minutes for CT pigs and between 20 and 60 minutes for PJ34 pigs. Data were expressed as mean ± standard error of the mean. *p less than 0.05 versus CT group; #p less than 0.05 versus respective basal.*

gases and acid-base data were maintained at PO_2 more than 100 mm Hg, PCO_2 less than 45 mm Hg and pH 7.3 ± 0.3.

Core Temperature

Core temperature was continually monitored by rectal thermometer using a YSI Model 44-TD tele-thermometer (Yellow Springs Instrument Co Inc, Yellow Springs, OH) and kept at 37°C.

Measurement of Infarct Size

The measurement and delineation of area at risk and infarct size we performed according to standard methods as described previously [14, 15]. Following 180 minutes reperfusion area at risk was delineated by religation of the left anterior descending artery and injection of monastryl blue pigment into the aorta. The area at risk was assessed by the weight of blue dyed myocardium and the total left ventricular mass weight. Immediately after the evaluation of area at risk, the heart slices were immersed into 1% triphenyl tetrazolium chloride (TTC, Sigma Chemical Co, St. Louis, MO) in phosphate buffer (pH 7.4) at 38°C for 20 minutes to determine myocardial infarct size. The area at risk and the infarct size were measured by computerized planimetry (Scion Image, Scion Corp, Frederick, MD) [14].

Immunohistochemical Analysis of Poly (ADP-Ribose) Synthesis in the Reperfused Myocardium

Heart samples were harvested at the conclusion of the experiments, formalin fixed and paraffin embedded. The PARS immunohistochemistry was performed as previously described [3]. Briefly, paraffin sections (3 μm) were deparaffinized in xylene and rehydrated in decreasing concentrations (100%, 95%, and 75%) of ethanol followed by 10 minutes incubation in PBS (pH 7.4). Sections were treated with 0.3% hydrogen peroxide for 15 minutes to eliminate endogenous peroxidase activity and then rinsed briefly in 10 mmol/L PBS. Nonspecific binding was blocked by incubating the slides for 1 hour in PBS containing 2% horse serum. Mouse monoclonal antipoly (ADP-ribose) antibody (Alexis, San Diego, CA) and isotype-matched control antibody were applied in a dilution of 1:100 for 2 hours at room temperature. After extensive washing (5 × 5 minutes) with PBS, immunoreactivity was detected with a biotinylated goat antirabbit secondary antibody and the avidin-biotin-peroxidase complex 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 $NiSO_4$ in 200 ml 0.1 mol/L acetate buffer). Sections were then counterstained with nuclear fast red, dehydrated and mounted in Permount. Photomicrographs were taken with a Zeiss Axiolab microscope (Carl Zeiss Inc, Thornwood, NY) equipped with a Fuji HC-300C digital camera (Fuji Photo Film Corp, Ltd, Tokyo, Japan).

Statistical Analysis

Data are expressed as mean ± standard error of the mean. Statistical significance was determined by repeated measures of analysis of variance with the group following a Bonferroni correction to adjust for the multiplicity of tests. Statistical significance is claimed at p less than 0.05.

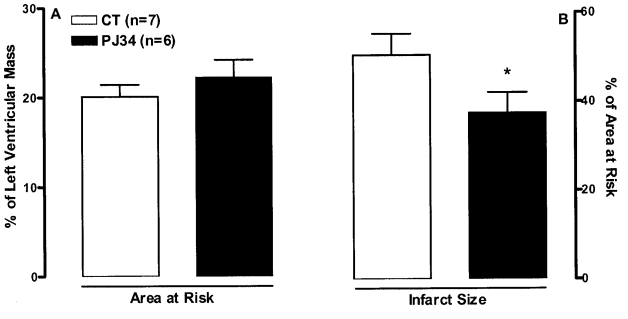

*Fig 3. (A) The area at risk of the left ventricle, (B) the infarct size in the area at risk after 60 minutes coronary artery occlusion and 180 minutes reperfusion in control pigs (CT) (open bars) and PJ34 pigs (filled bars). Values were expressed as mean ± standard error of the mean. *p less than 0.05 versus respective CT.*

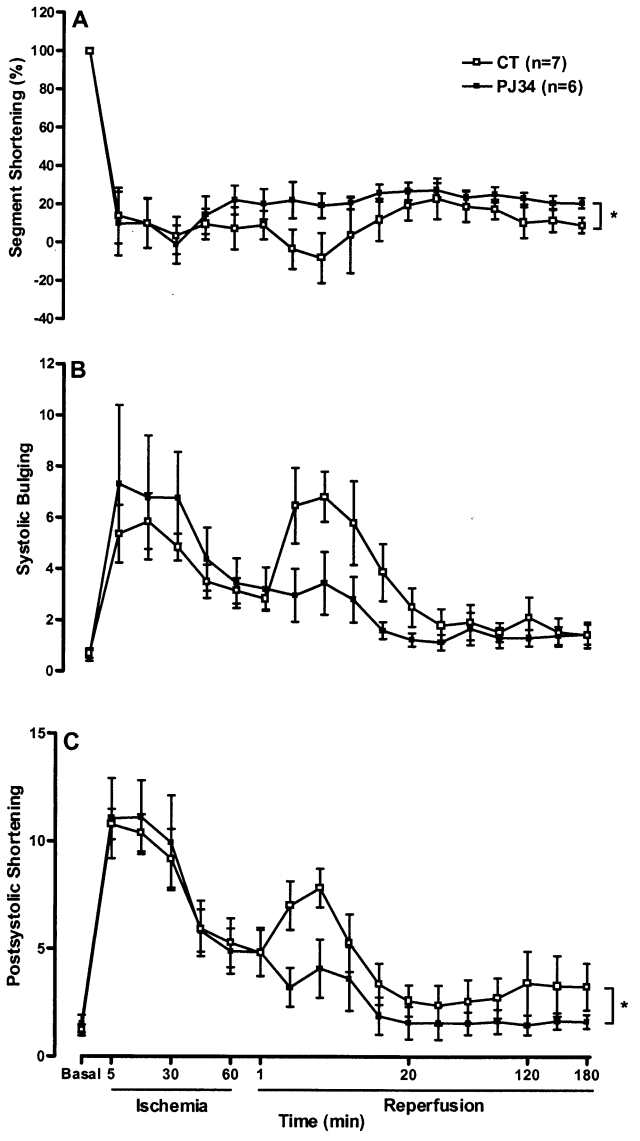

Fig 4. (A) Evolution of segment shortening, (B) systolic bulging, and (C) postsystolic shortening at basal during 60 minutes coronary artery occlusion and 180 minutes reperfusion in control pigs (CT) (□) *and PJ34 pigs* (■). *Values were expressed as mean ± standard error of the mean.* *p *less than 0.05 versus CT group.*

Results

Ventricular Fibrillation and Ventricular Arrhythmia

We experienced two ventricular fibrillations in each group during regional ischemia. The incidence of ventricular fibrillation was 28.6% in the control group and 33.3% in the PJ-34 group with no significant difference being observed between these two groups. Ventricular fibrillations developed at the time of 20 and 25 minutes regional ischemia in the control group, and at the time of 18 and 20 minutes in the PJ-34 group. All hearts were resuscitated by electrical cardioversion (75 J) with concomitant bolus injection of 1% lidocaine (1 to 2 mg/kg). No other arrhythmia was observed in this experiment.

Blood Gases, Hematocrit, and Core Temperature

The Pco_2 (35.2 ± 3.8 mm Hg) and Po_2 (381 ± 17 mm Hg) pressures in the CT group did not significantly differ from the PJ34 animals (37.3 ± 5.1 and 397 ± 15 mm Hg, respectively). Core temperature, hemoglobin concentration, and blood electrolytes analysis also showed no significant differences between both groups (data not shown).

Hemodynamic Measurements

No significant differences in hemodynamic measurements (MABP, left ventricular peak developed pressure, left ventricular end-diastolic pressure, heart rate, +dP/dt, and -dP/dt) were observed between CT and PJ34 animals during the stabilization period and ischemia. However, after 90 minutes of reperfusion heart rates had a significant difference (124 ± 8 beats/minute for CT and 144 ± 11 beats/minute for PJ34; $p < 0.05$, data not shown). At 180 minutes after reperfusion, +dP/dt was significantly different (1466 ± 217 mm Hg/second for CT and 2077 ± 259 mm Hg/second for PJ34; $p < 0.05$) (Fig 2 [top chart]).

In the CT group, coronary flow increased immediately upon reperfusion ($p < 0.05$) (Fig 2 [bottom chart]) with maximal flow observed between 5 and 10 minutes after the occluder was released. At these specific time points (5 and 10 minutes), the reflow values were significantly reduced in the PJ34 group ($p < 0.05$) (Fig 2 [bottom chart]). However, in the PJ34 animals, a gradual increase in reflow was observed, but this increase did not reach statistic significance when compared with basal value. After the hyperemic flow response, coronary blood flow gradually decreased at the end of experiment towards basal levels.

Area at Risk and Infarct Size

The occlusion of coronary artery (60 minutes) followed by reperfusion (180 minutes) did not affect the area at risk for both experimental groups (ranging from 20% to 22% of the left ventricular mass) (Fig 3A). However, in the PJ34 group the infarct size area (37.5% ± 4.5% of the area at risk) was significantly different from the CT group infarct size area (50.5% ± 4.8% of the area at risk; $p < 0.05$) (Fig 3B).

Regional Myocardial Function

No significant differences in percent of SS, systolic bulging, and postsystolic shortening were observed between groups neither at the end of stabilization period nor during the ischemic period. However, percentages of SS and PSS in PJ34 treated animals become significantly different during the reperfusion period. From 120 to 180 minutes during the reperfusion period, the myocardial function was significantly different between the groups. Percentage of SS was 10.8% ± 8.0%, 11.9% ± 6.0%, and 9.07% ± 3.67%, at 120, 150, and 180 minutes, respectively in the CT group during reperfusion, and 23.3% ± 3.1%, 21.1% ± 8.2%, and 21.1% ± 2.2%, at 120, 150, and 180 minutes, respectively in the PJ34 group during reperfusion ($p < 0.05$ vs CT, respectively) (Fig 4A). No significant

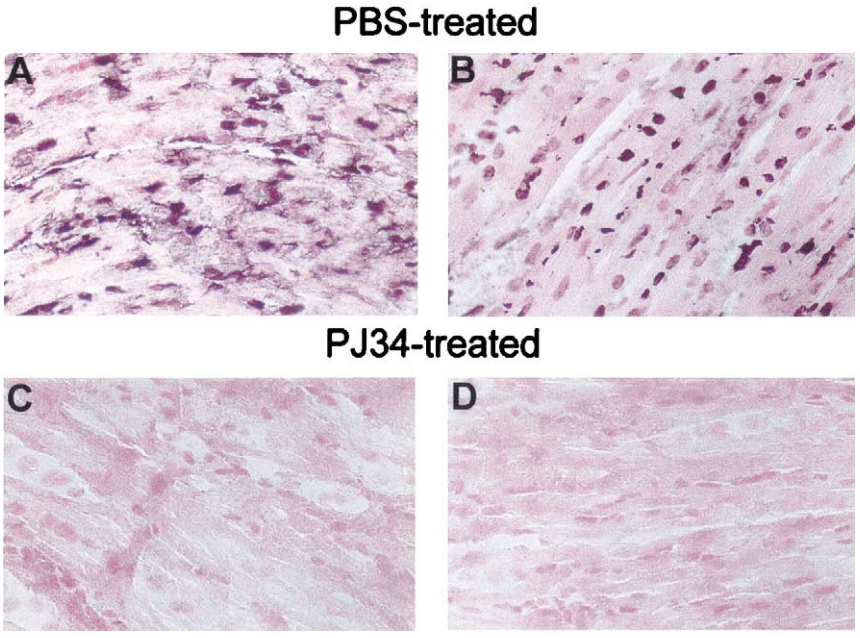

Fig 5. Immunohistochemical localization of PARS activation by immunohistochemical detection of poly (ADP-ribose) formation in left ventricle sections from pigs subjected to ischemia and reperfusion in the presence of (A) vehicle treatment and (B) control pigs (CT), and in (C) treated and (D) PJ34 pigs. (A) and (C) are the infarcted areas, and (B) and (D) are the areas at risk from the left ventricle after coronary artery occlusion in pigs. Note that poly (ADP-ribose) was undetectable in myocardium sections of pigs treated with PJ34. (PBS = phosphate buffered saline.) (Original magnification 400 ×. Counterstained with nuclear fast red.)

difference in systolic bulging was observed between the groups throughout 180 minutes reperfusion (Fig 4B). Postsystolic shortening was significantly reduced (from 3.3% ± 1.1% to 1.6% ± 0.3% for the CT and PJ34 groups, respectively; $p < 0.05$) (Fig 4C).

Poly (ADP-Ribose) Synthesis in the Myocardial Tissues

Myocardial sections from representative animals in both the CT and PJ34 treated groups are shown in Figure 5. An intense staining indicative of PARS activation was evident in the left ventricular free wall of the CT animals at the end of reperfusion, mostly located in the nuclei of cardiac myocytes (Figs 5A, 5B). Poly (ADP-ribose) was not detected in the nonischemic myocardium (data not shown). The staining was abolished in the left ventricle from pigs treated with PJ34, confirming the effectiveness of the agent in blocking PARS activity in the dosage regimen used (Figs 5C, 5D).

Comment

The main findings of this study show that the novel PARS inhibitor PJ34 provides myocardial protection by decreasing infarction size and improving postischemic regional and global myocardial function in a porcine model of ischemia-reperfusion. Many factors may influence the development of myocardial infarction, such as the duration of the experiment, the drug used for anesthesia, arterial pH, PO_2, and body temperature. Reperfusion of the myocardium is necessary to stop the infarction process. Recent studies demonstrated that inhibition of PARS prevents the functional alterations associated with ischemia-reperfusion injury in several organs, such as intestine [3], brain [16], and heart [9, 10, 17, 18]. The mechanisms for which inhibitors of PARS activity reduce infarct size in the heart and other organs are probably multiple. Previous studies have suggested that inhibition or inactivation of PARS, or both, modulates myocardial reperfusion injury by (1) prevention of oxidative stress formation-mediated energetic depletion [3], (2) inhibition of neutrophil infiltration into the reperfused and ischemic myocardium area [10], and (3) down-regulation of proinflammatory mediator production, such as tumor necrosis factor-α [18].

Many researchers had hypothesized that prevention of postischemic hyperemia during the initial reperfusion period by controlled flow might reduce the injury caused by reperfusion [19]. Our results demonstrate that treatment with PJ34 prevented the typical increase in coronary blood flow during the reperfusion period. This reduction on coronary blood flow during the reperfusion period induced by PJ34 may be related to reduction of infarct size. In fact, Halldorsson and colleagues [20], demonstrated in lungs that uncontrolled reperfusion results in a severe pulmonary reperfusion injury, and this injury is almost completely avoided by controlling the pressure during reperfusion. In another study, Sakamoto and coauthors [21] concluded that controlling initial perfusion pressure for 5 minutes attenuated ischemia-reperfusion injury.

Immunohistochemical detection indicated pronounced PARS activation in the area of necrosis and peri-infarct zone (latter zone is likely to coincide with the area at risk). Most of the staining was seen in cardiac myocytes, indicating that the heart tissue itself, rather than the infiltrating mononuclear cells, is the main site of PARS activation (and related pathophysiologic processes).

The PJ34 is a potent, phenanthridinone PARS inhibitor, which is approximately 10,000 times more potent than the prototypical PARS inhibitor 3-aminobenzamide. The PJ34 was previously evaluated in a cell-free PARS assay,

utilizing NAD^+ and purified PARS enzyme. The PJ34 dose-dependently inhibited PARS activity with an EC_{50} of 20 nmol/L. The EC_{50} of the prototypical PARS inhibitor 3-aminobenzamide was 200 μmol/L [13]. In order to evaluate the efficacy of PJ34 as a protector of cells against oxidant-induced necrosis, peroxynitrite-treated murine thymocytes were used. Both PJ34 and 3-aminobenzamide inhibited peroxynitrite-induced cell necrosis with respective EC_{50} values of 20 nmol/L and 30 μmol/L [13]. The reduction in poly (ADP-ribose) staining in the PJ34 treated animals directly demonstrates that the dosing regimen of the PARS inhibitor is sufficient to suppress PARS activation in the heart. Because PARS activation triggers cellular necrosis due to cellular energetic collapse [22], we believe that the primary mode of PARS inhibitor's protective effects is related to a direct inhibition of myocyte necrosis in myocardial reperfusion. The peri-infarct zone (area at risk) contains viable cells in which PARS is markedly activated, and is the likely site of the PARS inhibitor's beneficial effects in vivo. Based on its high potency and the direct evidence of reduction in poly (ADP-ribose) staining in the reperfused myocardium, we can assume that PJ34 was able to fully suppress PARS activity in the myocardium. This indicates the likelihood that the extent of suppression of infarct size (approximately 30%) is the maximal suppression that can be expected from this class of agents. This degree of suppression is consistent with the degree of suppression in previous studies using PARS deficient animals [10, 23]. Because PARS is only one of the many downstream processes initiated by free radical burst during reperfusion injury, it is conceivable that appropriately selected combination therapies that include PARS inhibitors as one component will be able to provide additional benefit.

Taken together, this study directly demonstrates the prolonged and confined activation of PARS in the reperfused myocardium and its inhibition by PJ34, and also demonstrates that a potent pharmacological inhibition of PARS provides both morphologic and functional improvements in a porcine model of myocardial reperfusion injury. These observations indicate that the concept of pharmacological inhibition of PARS should be further explored in the context of experimental therapy of heart attacks.

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INVITED COMMENTARY

This article describes a very well conducted animal study on the protective effects of a new poly (ADP-ribose) synthetase (PARS) inhibitor, PJ34, during myocardial ischemia and reperfusion injury. Dr Faro and coauthors can be congratulated on an excellent study using state of the art cardiovascular modalities and an equally well written manuscript. This study shows without a doubt that PARS inhibitors play a significant role in cell injury following ischemia-reperfusion and clinical studies are indicated following the excellent outcome of this experiment.

Poly (ADP-ribrose) synthetase is a nuclear enzyme that has been extensively investigated. It has been long known that PARS plays a significant role in apoptosis, but the exact mechanism by which it causes cell death, cellular differentiation, malignant transformation, gene amplification, and DNA replication is still being heavily investigated. During the 1970s and 1980s PARS received tremendous attention and it was found that PARS activation leads to NAD^+ overuse that eventually causes intracellular ATP depletion. Glycolysis and mitochondrial respiration subside leading to necrotic type of cell death. Researchers interest in PARS's role in this suicidal cell death gained new momentum in the mid-nineties when it was found that nitrous oxide, produced from L-Arginine by nitrous oxide synthetase, was an important mediator in inflammatory and ischemia-reperfusion injury and that PARS activation played a significant role in that cascade. It is equally important from a research standpoint that PARS inhibitors are available making it easier to delineate the physiologic role of PARS. The three most common are nicotinamide, 3-aminobenzamine, and benzamine. It has been proven without a doubt that DNA single-strand break is the most likely activator of PARS during ischemia-reperfusion. It is not known whether a massive single DNA strand break activates the system by itself, or whether a minor injury initiates a self-sustaining cascade, which could include neutrophil activation, adhesion molecules up-regulation or yet an unknown positive "feed-forward" cycle that results in slow apoptosis. Although, there seems no doubt that PARS plays an important role in caspase-3 mediated apoptotic cell death, several researchers have noted that this process is too slow to explain the necrotic cell death that occurs during ischemia-reperfusion. Energy is depleted early in ischemia-reperfusion injury making it unlikely that energy consuming process like apoptosis can occur. Based on these findings many feel that PARS plays more of a role as a facilitator of oxygen free radicals, oxidant induced cell necrosis, and leukocyte induced injury. It is somewhat surprising that PARS has not been deleted during the evolutionary process because of its significant suicidal effects. Some scientists have postulated that PARS plays more of a role in DNA maintenance and repair than previously thought. On the other hand it does not seem to be necessary for normal development, since three recently developed PARS "knockout" animal models have all been morphologically and physiologically normal and viable. These "knock out" models are going to be invaluable in evaluating the short and long term effect of PARS inhibition.

Although, the natural and pathological function of PARS is still being evaluated and many aspects of this fascinating enzyme are unknown, this study and others show that inhibiting its function plays a significant role in decreasing ischemia-reperfusion injury in the myocardium. This new PARS inhibitor, PJ34, has the advantage of being more potent than the ones previously used and have been thoroughly investigated in both cell free PARS assays and cell cultures. This study also shows nicely that this PARS inhibitor causes complete enzyme inhibition. Therefore, making it more unlikely that the infarct size can be suppressed more than 30% with higher dosage or different inhibitor. If PJ34 performs as well in human studies as in animals without any unforeseen complications, we can expect a one-third decrease in infarct size. Using PARS inhibitors does not prevent us from blocking other "down stream"processes initiated by free radical bursts during reperfusion , thereby decreasing the infarct size even further. Also of interest is that several studies have shown that PARS activation after ischemia-reperfusion in the myocardium is prolonged making it possible to get positive effects from giving PARS inhibitors like PJ34 even several hours after the initial ischemic event. This study has opened the doors to human studies into the effect of PARS inhibitors after ischemia-reperfusion.

Ari O. Halldorsson, MD

Division of Cardiothoracic Surgery
Texas Tech University Health Sciences Center
3601 4th St, Suite 3A111
Lubbock, TX 79430
e-mail: ari.halldorsson@ttmc.ttuhsc.edu.

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