
Poly(ADP-Ribose) Polymerase Inhibition Improves Postischemic Myocardial Function after Cardioplegia-Cardiopulmonary Bypass

Tanveer A Khan, MD, Marc Ruel, MD, MPH, Cesario Bianchi, MD, PhD, Pierre Voisine, MD, Katalin Komjáti, MD, PhD, Csaba Szabo, MD, PhD, Frank W Sellke, MD, FACS

BACKGROUND: Poly(ADP-ribose) polymerase activation has been shown to contribute to the pathogenesis of myocardial ischemia-reperfusion injury. We hypothesized that a novel poly(ADP-ribose) polymerase inhibitor, INO-1001, provides myocardial protection and improves cardiac function after regional ischemia and cardioplegia-cardiopulmonary bypass (CPB).

STUDY DESIGN: Pigs were subjected to 30 minutes of regional ischemia by distal left anterior descending coronary artery ligation followed by CPB (60 minutes) with hyperkalemic cardioplegia (45 minutes). The myocardium then was reperfused post-CPB for 90 minutes. After 15 minutes of ischemia, the treatment group ($n = 6$) received an INO-1001 bolus (1mg/kg) before a continuous infusion (1mg/kg/hour). Control pigs ($n = 6$) received vehicle solution. Left ventricular pressure was monitored, from which the maximum, positive first derivative of left ventricular pressure over time ($+dP/dt$) was calculated. Regional myocardial function in the ischemic area was determined by sonomicrometric analysis. Infarct size was measured as the percent of the ischemic area by tetrazolium staining. Myocardial sections were immunohistochemically stained for poly(ADP-ribose) as a measure of poly(ADP-ribose) polymerase activity and inhibition.

RESULTS: Pigs treated with INO-1001 showed improvements in the $+dP/dt$ at 60 and 90 minutes of post-CPB reperfusion (both $p = 0.03$) and percent segmental shortening at 30, 60, and 90 minutes of post-CPB reperfusion ($p = 0.03, 0.009,$ and 0.03 , respectively). Infarct size was decreased in the treatment group ($18.5 \pm 5.7\%$ versus $52.0 \pm 7.7\%$, INO-1001 versus control, $p = 0.03$). Poly(ADP-ribose) was reduced in myocardial sections from INO-1001-treated animals compared with controls.

CONCLUSIONS: These results suggest that INO-1001 provides myocardial protection by reducing the extent of infarction and improves cardiac function after regional ischemia and cardioplegia-CPB. (*J Am Coll Surg* 2003;197:270–277. © 2003 by the American College of Surgeons)

Drs Csaba Szabo and Katalin Komjáti have a financial relationship with the Inotek Pharmaceuticals Corporation.

Funding was provided by grants from the National Institutes of Health, NIH R01 HL46716-07 and NIH R43 HL-65863. Dr Khan is supported by an Individual National Research Service Award from the National Institutes of Health, NIH NRSA 1F32 HL69651-01.

Abstract presented at the American College of Surgeons 88th Annual Clinical Congress, Surgical Forum, San Francisco, CA, October 2002.

Received November 27, 2002; Revised February 24, 2003; Accepted March 11, 2003.

From the Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA (Khan, Ruel, Bianchi, Voisine, Sellke) and Inotek Corporation, Beverly, MA (Komjáti, Szabo).

Correspondence address: Frank W Sellke, MD, FACS, Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, 110 Francis St, LMOB 2A, Boston, MA 02215.

Surgical revascularization for treatment of myocardial ischemia restores vital blood flow but is accompanied by reperfusion injury. Many cardiac surgical procedures continue to be performed with cardioplegia-cardiopulmonary bypass (CPB), which produces an inflammatory response that potentially results in organ dysfunction, including decreased myocardial performance. Postoperative myocardial dysfunction from reperfusion injury and cardioplegia-CPB increases the risk of morbidity and mortality despite successful revascularization. Reperfusion injury after ischemic insult to myocardial tissue is, in part, mediated by the generation

Abbreviations and Acronyms

| | |
|------|--|
| ABG | = arterial blood gas |
| CPB | = cardiopulmonary bypass |
| ICAM | = intercellular adhesion molecules |
| LAD | = left anterior descending coronary artery |
| LV | = left ventricular |
| NAD | = nicotine adenine dinucleotide |
| PARP | = poly(ADP-ribose) polymerase |

of reactive oxygen and nitrogen species such as hydroxyl radicals ($\text{OH}\cdot$), hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and peroxynitrite (ONOO^-). Reactive oxygen and nitrogen species produce single-strand breaks in DNA.¹ Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme that is activated by single-strand breaks in DNA from oxidant stress and has been implicated in DNA repair. This chromatin-bound enzyme catalyzes the synthesis of poly(ADP-ribose) from nicotine adenine dinucleotide (NAD). In acute myocardial ischemia and the consequent oxidant stress, persistent activation of PARP from oxidative damage of DNA strands results in the consumption of NAD.² Diminished intracellular levels of NAD prevent efficient synthesis of adenosine triphosphate (ATP). Depletion of these critical intracellular energy stores results in cellular dysfunction and, ultimately, cell death.

PARP inhibition has been shown to reduce oxidant injury to cultured myocardial cell lines exposed to hydrogen peroxide.^{3,4} In isolated, perfused heart models, blockade of PARP activity by chemical inhibition or genetic deletion of the enzyme was associated with decreased infarct size and enhanced myocardial function after ischemia and reperfusion.^{4,5} These findings were supported by studies using *in vivo* models in the rat that showed inhibitors of PARP decrease infarct size after reperfusion injury.^{6,7} PARP inhibition has been shown to also provide myocardial protection in pig models of reperfusion injury. Treatment with the prototypical agent 3-aminobenzamide produced both a diminished area of left ventricular (LV) infarction and reduced post-ischemic contractile dysfunction.⁸ Recently, a study from our laboratory demonstrated that intravenous administration of PJ34, a novel phenanthridone PARP inhibitor, in a porcine model of regional myocardial ischemia and reperfusion resulted in diminished infarct size and improved cardiac function.⁹ But the role of PARP in the pathogenesis of myocardial injury associated with

cardioplegia-CPB has not yet been investigated. In this study, we assessed the hypothesis that treatment with a novel PARP inhibitor, INO-1001, provides myocardial protection and improves cardiac function in a model of regional ischemia and cardioplegia-CPB.

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 US National Institutes of Health guidelines regulating the care and use of laboratory animals (NIH publication No. 5377-3, 1996).

Experimental Design

Pigs (35 to 40 kg) were divided randomly into control ($n = 6$) and treatment ($n = 6$) groups. Both groups were subjected to regional left ventricular (LV) ischemia by ligation of the distal left anterior descending (LAD) coronary artery, beyond the first diagonal branch, using a Ramel tourniquet for 30 minutes before the initiation of CPB. Regional ischemia was confirmed by the observation of regional cyanosis of the distal LAD territory on the myocardial surface¹⁰ and the measurement of distal coronary blood flow. After 30 minutes of regional ischemia, pigs underwent CPB for 60 minutes. After 5 minutes of CPB, an aortic crossclamp was placed and the heart was arrested with hyperkalemic cardioplegia for 45 minutes. The aortic crossclamp and LAD ligation then were removed and the myocardium was reperfused for 10 minutes on CPB. Pigs were weaned from CPB, and the myocardium then was reperfused post-CPB for 90 minutes until completion of the experiment.

The treatment group received an intravenous (IV) bolus of the ultrapotent PARP inhibitor INO-1001 (1 mg/kg; Inotek Corporation, Beverly, MA) 15 minutes after distal LAD ligation. The IV bolus was immediately followed by a continuous IV infusion (1mg/kg/hour), which was continued during the remaining periods of regional ischemia, cardioplegia-CPB, and post-CPB reperfusion. The control group received vehicle solution as a bolus followed by a continuous infusion. Arterial pressure, LV pressure, coronary blood flow, heart rate, EKG, oxygen saturation, and temperature were moni-

tored continuously throughout the experiment. Hemodynamic variables were acquired and analyzed using a digital measurement system (Sonometrics Corporation, London, Ontario, Canada). Arterial blood gases were monitored every 30 minutes and as needed using a blood gas analyzer (AVL Scientific Corp, Roswell, GA). Arterial blood gas (ABG) parameters were maintained according to the following: pH, 7.35 to 7.45; PCO₂, 35 to 45 mmHg; PO₂, 100 to 200 mmHg; and HCO₃⁻, 22 to 28 mEq/L.

Surgical procedure

Pigs were anesthetized with intramuscular ketamine hydrochloride (20 mg/kg) and xylazine (15 mg/kg). General anesthesia with isoflurane gas was maintained throughout the experiment by endotracheal intubation. A volume-cycled ventilator (Harvard Apparatus, South Natick, MA) was used for mechanical ventilation, which was turned off during cardioplegia-CPB. Core temperature was monitored with a rectal thermometer (Yellow Springs Instrument Company, Inc, Yellow Springs, OH) and maintained at 37°C. The right internal jugular vein was cannulated for intravenous access and venous blood sampling. The right common carotid artery was cannulated for arterial blood sampling and intraarterial blood pressure monitoring using a catheter-tipped manometer (Millar Instruments, Houston, TX). A median sternotomy was performed, and the pericardial sac was exposed and opened. A catheter-tipped manometer was placed through a purse-string stitch with 4-0 Prolene suture (Ethicon, Inc, Somerville, NJ) in the LV apex for LV pressure monitoring. Four 2-mm digital ultrasonic probes (Sonometrics Corporation, London, Ontario, Canada) were placed in the subepicardial layer of the distal LAD territory of the LV for analysis of regional myocardial function. A 2-0 silk suture (Ethicon, Inc, Somerville, NJ) was passed around the LAD distal to the first diagonal branch and controlled with a Ramel tourniquet for ligation. A 2-mm ultrasonic flow probe (Transonic Systems Inc, Ithaca, NY) was placed around the LAD distal to the site of ligation for measurement of coronary blood flow. Pigs were given intravenous heparin (300 U/kg) and cannulated with an aortic cannula through the distal ascending aorta through double purse-string stitches with 4-0 Prolene sutures and a single-stage, venous cannula through the right atrium with a single purse-string stitch with 4-0 Prolene suture. CPB was initiated with a kaolin-activated clotting time

of more than 480 seconds, which was maintained with repeat administrations of intravenous heparin. The proximal aorta was crossclamped and cold, crystalloid cardioplegia was infused into the aortic root through an 18-g catheter. An initial 300 mL of high potassium cardioplegia was administered to each animal followed by an additional 150 mL of low potassium cardioplegia each 15 minutes (total volume administered 600 mL).

Measurement of global and regional myocardial function

Global myocardial function was assessed by calculating the maximum, positive first derivative of LV pressure over time (+dP/dt) using Cardiosoft software (Sonometrics Corp, London, Ontario, Canada). Regional myocardial function was assessed by a sonometric digital ultrasonic measurement system (Sonometrics Corp, London, Ontario, Canada) using four 2-mm digital ultrasonic probes implanted in the subepicardial layer approximately 10 mm apart within the ischemic LV area and secured to the epicardium with a "U stitch" using 6-0 Prolene suture. Cardiosoft software also was used for data analysis to determine regional myocardial function. Measurements were taken at baseline; after 30 minutes of regional ischemia; and after 30, 60, and 90 minutes of post-CPB reperfusion. The ventilator was stopped during data acquisition to eliminate the effects of respiration. Measurements were made during at least three cardiac cycles in normal sinus rhythm and then averaged. Regional contractility was assessed by calculating percent segmental shortening. Digital data were inspected for the correct identification of end-diastole and end-systole. End-diastolic segment length (EDL) was measured at the onset of the positive dP/dt, and the end-systolic segment length (ESL) at the peak negative dP/dt.

Determination of myocardial infarct size

The measurement of infarct size was performed as previously described.¹⁰ After 90 minutes of post-CPB reperfusion, the ischemic area was determined by religation of the distal LAD and injection of monastryl blue pigment (Engelhard Corp, Louisville, KY) at a 1:5 dilution in PBS into the aortic root after replacement of the aortic crossclamp. The heart then was rapidly excised and divided into 1-cm transverse sections. These myocardial sections were immersed in 1% triphenyl tetrazolium chloride (TTC, Sigma Chemical Co, St Louis, MO) in

phosphate buffer (pH 7.4) at 38°C for 20 minutes. The ischemic area and infarct size were measured by computerized planimetry (Scion Image, Scion Corp, Frederick, MD). The ischemic area and infarct size then were calculated by the weight of the myocardium not stained by blue dye and TTC, respectively, as a percentage of the total LV weight.

Immunohistochemical detection of poly(ADP-ribose)

Paraffin-embedded myocardial tissue sections were deparaffinized in xylene and rehydrated in decreasing concentrations (100%, 95%, and 70%) of ethanol followed by a 5-minute incubation in PBS. Sections were treated with 0.3% hydrogen peroxide for 15 minutes to block endogenous peroxidase activity and then rinsed briefly in PBS. Nonspecific binding was blocked by incubating the slides for 1 hour in 0.25% Triton X/PBS containing 2% horse serum. To detect poly(ADP-ribose), a routine immunohistochemical procedure was carried out as previously described⁸ with minor modifications as follows. Mouse monoclonal anti-poly(ADP-ribose) antibody or isotype-matched control antibody was applied in a dilution of 1:200 for 1 hour at room temperature. After extensive washing (three washings of 10 minutes each) with 0.25% Triton X/PBS, immunoreactivity was detected with a biotinylated horse anti-mouse secondary antibody and the avidin-biotin-peroxidase complex (ABC), both supplied in the Vector Elite kit (Vector Laboratories, Burlingame, CA). Color was developed using a Ni-DAB substrate kit. Sections then were briefly rinsed in tris/saline (pH 7.6) and incubated in tris/cobalt (pH 7.2) for 2 minutes. Sections then were counterstained with nuclear fast red, dehydrated, and mounted. Photomicrographs were taken. All histologic and immunohistochemical samples were examined by an investigator in a blinded fashion.

Statistical analysis

Values are shown as the mean \pm SEM. Statistical testing was performed using the Mann-Whitney test or analysis of variance (ANOVA). Statistical significance was accepted as $p < 0.05$.

RESULTS

Hemodynamic parameters, ABG, and temperature

No significant differences in heart rate were observed between the groups during the experimental protocol except at 90 minutes of post-CPB reperfusion, when the

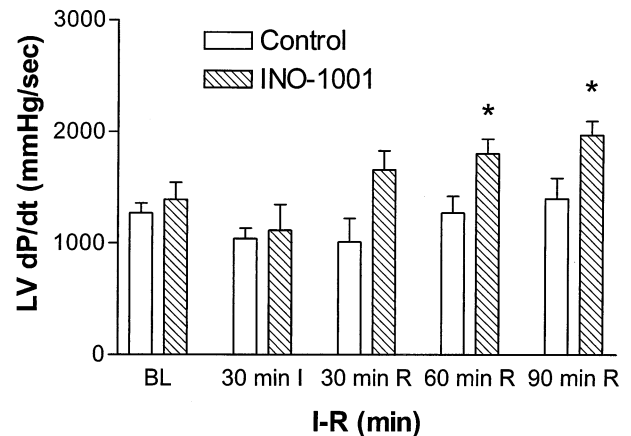


Figure 1. Global myocardial function. Left ventricular (LV) function as measured by $+dP/dt$ was significantly improved in the INO-1001 group compared with the control group at 60 and 90 minutes of postcardioplegia–cardiopulmonary bypass (CPB) reperfusion ($*p < 0.05$). The $+dP/dt$ also was increased at 30 minutes of reperfusion, but was not quite statistically significant ($p = 0.05$). BL, baseline; I, ischemia; R, post-CPB reperfusion.

rate in the INO-1001 group exceeded that in the control group (124.7 ± 3.0 beats/minute versus 102.5 ± 6.0 beats/minute, INO-1001 versus control, $p = 0.03$). At 60 and 90 minutes of post-CPB reperfusion, mean arterial pressure was greater in the INO-1001 group (72.3 ± 3.4 mmHg versus 47.3 ± 6.6 mmHg and 66.8 ± 5.7 mmHg versus 46.4 ± 5.3 mmHg, respectively, INO-1001 versus control, both $p < 0.05$). Mean ABG parameters including pH, PCO_2 , and PO_2 and core temperature were not significantly different between treatment and control groups.

Global myocardial function

The maximum positive first derivative of LVP over time ($+dP/dt$) was used as a measure of global LV function (Fig. 1). Pigs treated with INO-1001 showed significant improvements in $+dP/dt$ at 60 and 90 minutes of post-CPB reperfusion compared with controls ($1,803 \pm 133$ mmHg/second versus $1,271 \pm 151$ mmHg/second and $1,969 \pm 127$ mmHg/second versus $1,398 \pm 186$ mmHg/second, respectively, INO-1001 versus control, both $p = 0.03$). After 30 minutes of post-CPB reperfusion, a trend toward increased $+dP/dt$ compared with the control group was observed ($1,658 \pm 170$ mmHg/second versus $1,012 \pm 209$ mmHg/second, INO-1001 versus control, $p = 0.05$). There were no significant differences between the groups in LV $+dP/dt$ at baseline and after 30 minutes of ischemia.

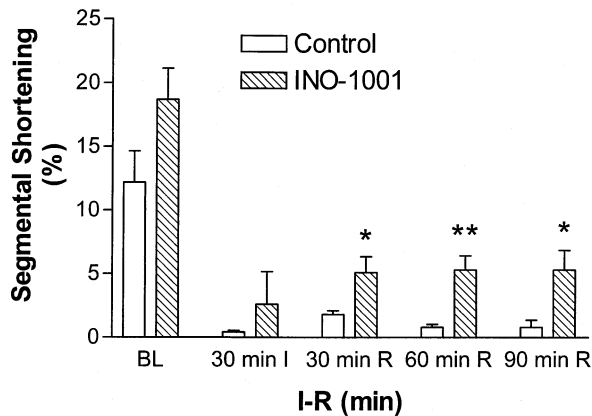


Figure 2. Regional myocardial function. Percent segmental shortening was determined through placement of sonomicrometric crystals in the ischemic area of the left ventricle. Percent segmental shortening was significantly greater in the animals treated with INO-1001 compared with that in control animals during the period of postcardioplegia–cardiopulmonary bypass (CPB) reperfusion at 30, 60, and 90 minutes (* $p < 0.05$, ** $p < 0.01$). BL, baseline; I, ischemia; R, post-CPB reperfusion.

Regional myocardial function

Segmental shortening as determined from sonometric crystal data analysis was used as a measure of regional myocardial function in the ischemic, distal LAD territory (Fig. 2). The percent segmental shortening significantly improved after 30 minutes ($5.1 \pm 1.3\%$ versus $1.8 \pm 0.3\%$, INO-1001 versus control, $p = 0.03$), 60 minutes ($5.3 \pm 1.1\%$ versus $0.8 \pm 0.2\%$, INO-1001 versus control, $p = 0.009$), and 90 minutes ($5.3 \pm 1.5\%$ versus $0.8 \pm 0.6\%$, INO-1001 versus control, $p = 0.03$) of post-CPB reperfusion in the INO-1001-treated animals compared with controls. No significant differences were observed between the INO-1001 group and control group at baseline and after 30 minutes of ischemia.

Coronary blood flow

No difference in LAD blood flow between the INO-1001 and control groups was apparent at baseline (Fig. 3). At 30 minutes of post-CPB reperfusion, coronary blood flow in the INO-1001-treated animals was significantly attenuated compared with the increased flow in the control animals, consistent with a hyperemic response to ischemia (35.62 ± 7.614 mL/minute versus 82.63 ± 12.32 mL/minute, INO-1001 versus control, $p = 0.02$). At 60 and 90 minutes of post-CPB reperfusion, the elevated LAD blood flow in the control group decreased to flow rates similar to those of the INO-1001 group, with no significant difference between them.

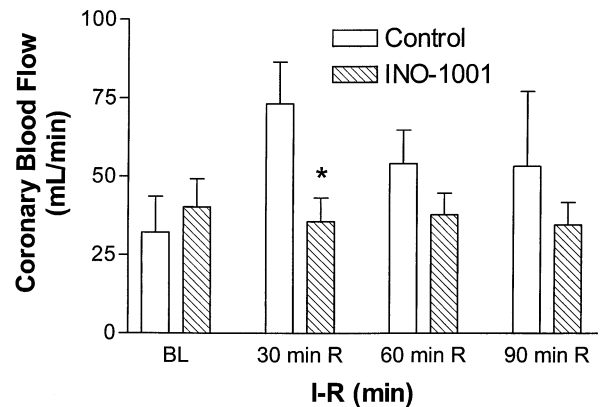


Figure 3. Coronary blood flow. Early in the postcardioplegia–cardiopulmonary bypass (CPB) reperfusion period at 30 minutes, the increase in coronary blood flow observed in the control group, consistent with a hyperemic response to ischemic injury, was attenuated in the INO-1001 group. Coronary blood flow then decreased in the control group to levels similar to those in the INO-1001 group at 60 and 90 minutes of post-CPB reperfusion (* $p < 0.05$). BL, baseline; I, ischemia; R, post-CPB reperfusion.

Myocardial infarct size

No significant difference was observed between the LV ischemic areas of the INO-1001 and control groups. In animals treated with INO-1001, the myocardial infarct size, measured as the percent of the ischemic area, was significantly less than the infarct size in control animals ($18.5 \pm 5.7\%$ versus $52.0 \pm 7.7\%$, INO-1001 versus control, $p = 0.03$; Figs. 4 and 5).

Immunohistochemistry

Regional ischemia and cardioplegia-CPB resulted in a marked activation of PARP, as evidenced by the accumulation of poly(ADP-ribose) in the myocardial nuclei in the LV ischemic area. INO-1001 treatment abolished poly(ADP-ribose) formation in ischemic myocardial tissue (Fig. 6).

DISCUSSION

In our model of regional ischemia and cardioplegia-CPB, we demonstrated that treatment with INO-1001, a novel PARP inhibitor, improves global and regional myocardial function and decreases LV infarct size. Myocardial protection in the INO-1001 group was apparent in the attenuation of increased coronary blood flow early in the post-CPB reperfusion period associated with the hyperemic response to ischemic injury. These results are consistent with our previous findings in a study of PJ34, a potent earlier-generation PARP inhibitor. In the pig

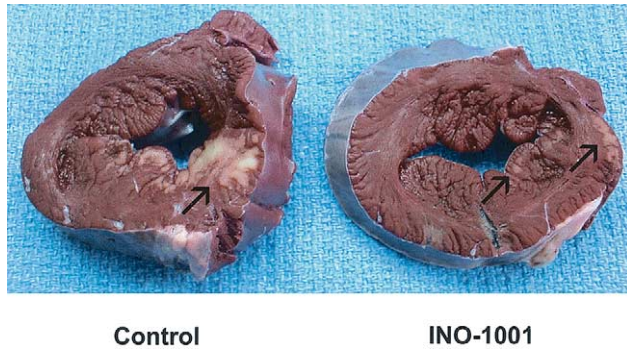


Figure 4. Representative image of left ventricular (LV) infarct size determined by triphenyl tetrazolium chloride (TTC) staining. Infarct size was markedly decreased in the LV myocardium of INO-1001-treated animals (right) compared with controls (left).

model of regional myocardial reperfusion injury only, PJ34 administration improved the $+dp/dt$ and percent segmental shortening, and decreased LV infarct size.⁹ In the pig model of regional ischemia and cardioplegia-CPB in this study, we demonstrated that INO-1001 improves cardiac function and provides myocardial protection, which has implications in the prevention of reperfusion injury after surgical revascularization with extracorporeal circulatory support. Preservation of cellular energy is a mechanism by which PARP inhibition likely contributes to the prevention of myocardial reperfusion injury. Excessive activation of PARP may result from generation of radical oxygen and nitrogen species that has been shown during CPB.^{11,12} PARP activation caused by DNA damage from the oxidative stress then

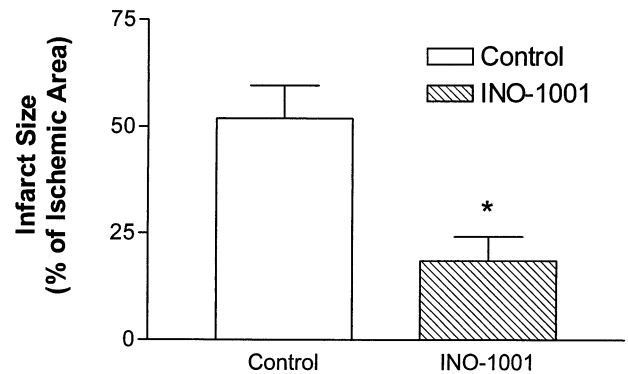


Figure 5. Quantification of left ventricular (LV) infarct size. LV infarct size was measured as the percent of the LV ischemic area. In the INO-1001 group, the percent of the LV ischemic area that progressed to infarction was significantly reduced compared with that in control animals (* $p < 0.05$).

leads to consumption of energy intermediates, NAD and ATP, with potential for cellular dysfunction and death.^{1,2} Yet, other mechanisms may have significant roles in the myocardial protective effect of PARP inhibition in reperfusion injury.

Surgical myocardial revascularization with CPB is associated with reperfusion injury and a systemic inflammatory response that contribute to postoperative myocardial dysfunction. Leukocyte activation has been suggested to contribute to the inflammatory injury associated with CPB.¹³ Low affinity interactions between neutrophils, platelets, and vascular endothelial cells are mediated by selectins, L-selectin on leukocytes, P-selectin on platelets, and E-selectin on endothelial

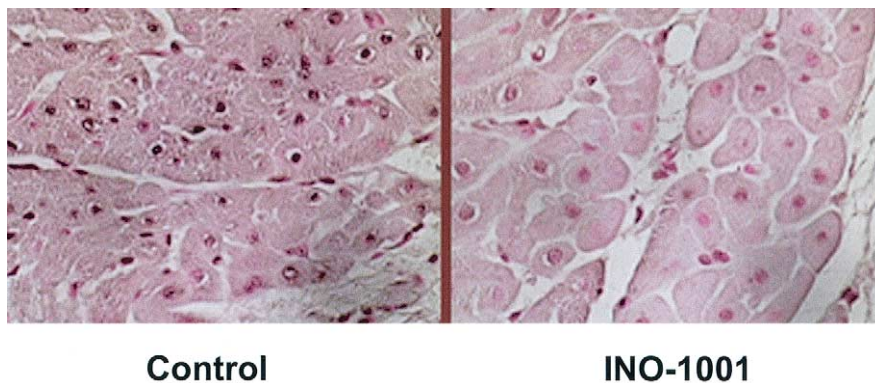


Figure 6. Immunohistochemical detection of poly(ADP-ribose). Poly(ADP-ribose) accumulation as a result of regional ischemia and cardioplegia-cardiopulmonary bypass (CPB) was ameliorated by INO-1001. The left panel shows a representative myocardial section (ischemic area) from a vehicle-treated, control animal. The dark staining in the nuclei indicates poly(ADP-ribose) accumulation, ie, poly(ADP-ribose) polymerase activation. INO-1001 treatment (right panel) diminishes poly(ADP-ribose) accumulation, consistent with its mode of action.

cells. CD11b/CD18 integrin on the leukocyte cell surface binds intercellular adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM) on the endothelial cell surface to produce firm adhesion that leads to endothelial transmigration. Neutrophils then release oxygen free radicals, proteases, and proinflammatory mediators in the interstitial space that cause oxidative damage, fluid extravasation, and cellular dysfunction and death. Treatment with monoclonal antibodies against L-selectin, P-selectin, and ICAM-1 has been shown to decrease reperfusion injury in animal models.¹⁴⁻¹⁶

Neutrophil activation during CPB has been demonstrated in numerous clinical studies.^{17,18} The use of leukocyte depletion filters may reduce myocardial reperfusion injury in patients undergoing CABG or valve replacement with CPB.^{19,20} The mechanism of action of PARP inhibition in reducing reperfusion injury may involve modulation of the interaction between leukocytes and endothelial cells. In a study using mice lacking a functional gene for PARP, reduced endothelial expression of P-selectin and ICAM-1 resulted in diminished neutrophil infiltration and myocardial reperfusion injury.²¹ Similarly, microvascular reperfusion injury of the liver was diminished in PARP knockout mice in which adhesion molecule expression and leukocyte-endothelial cell interactions were reduced.²² Still, other mechanisms that are not related to leukocyte interaction with endothelial cells likely contribute to reperfusion injury. In a study of ICAM-1 and P-selectin knockout mice, no significant difference was observed in infarct size after ischemia and reperfusion between the wild type and adhesion molecule-deficient mice.²³ PARP activity also has been demonstrated to be involved in the regulation of nuclear transcription factor NF- κ B in studies using PARP knockout mice.²⁴ But the ability of pharmacologic PARP inhibition to augment NF- κ B activation appears to be dependent on the experimental system used.²⁵

INO-1001 is approximately 10 times more potent on isolated enzyme and in cytoprotection assays than the earlier-generation phenanthridone PARP inhibitor PJ-34.²⁶ INO-1001 exerts protective effects in various experimental models of injury that are known to be mediated by PARP activation, such as stroke.²⁶ There are multiple studies demonstrating that PARP inhibition or PARP deficiency exert cardioprotective effects, including studies on myocardial infarction (see above), cardiac

transplantation,²⁷ diabetic cardiomyopathy,²⁸ ischemic cardiomyopathy,²⁹ cardiac dysfunction associated with endotoxic shock,³⁰ and cardiomyopathy elicited by cardiotoxic anticancer agents.³¹ This study is the first one to implicate PARP activation in the pathogenesis of CPB. In the clinical arena, CPB is frequently associated with remote organ injury, most notably neurologic dysfunction.³² Because this condition is thought to involve CNS ischemia and N-methyl-D-aspartate (NMDA) receptor activation,³² and PARP inhibition is protective in various forms of neuronal ischemia and reperfusion injury that are dependent on the above mechanisms,³³ it is tempting to hypothesize that PARP inhibition may beneficially affect CNS function and other forms of remote organ injury caused by CPB. This question will be a subject of further investigations.

In conclusion, we demonstrated that administration of the novel PARP inhibitor, INO-1001, provides myocardial protection and improves postischemic cardiac function in our model of regional ischemia and cardioplegia-CPB. In this study, we used an intravenous route for delivery of the agent; another potential route for administration is in the cardioplegia solution. A study to determine if this route provides additional myocardial protection may be a valuable future investigation considering the increasing numbers of patients undergoing cardiac surgery who are considered high-risk and are more likely to develop postoperative myocardial dysfunction.

Author contributions

Study conception and design: Khan, Ruel, Bianchi, Szabo, Sellke

Acquisition of data: Khan, Ruel, Bianchi, Komjáti, Szabo

Analysis and interpretation of data: Khan, Ruel, Bianchi, Voisine, Szabo, Sellke

Drafting of manuscript: Khan

Critical revision: Khan, Bianchi, Voisine, Szabo, Sellke

Statistical expertise: Khan, Ruel, Voisine

Obtaining funding: Khan, Ruel, Bianchi, Szabo, Sellke

Supervision: Khan, Ruel, Bianchi, Sellke

Acknowledgment: We would like to thank Catherine Grant at the Beth Israel Deaconess Medical Center Animal Research Facility for her expertise in technical assistance.

REFERENCES

- Schraufstatter IU, Hinshaw DB, Hyslop PA, et al. Oxidant injury of cells. DNA strand-breaks activate polyadenosine diphosphate-ribose polymerase and lead to depletion of nicotinamide adenine dinucleotide. *J Clin Invest* 1986;77:1312–1320.
- Szabo C, Zingarelli B, O'Connor M, et al. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. *Proc Natl Acad Sci USA* 1996;93:1753–1758.
- Gilad E, Zingarelli B, Salzman AL, et al. Protection by inhibition of poly (ADP-ribose) synthetase against oxidant injury in cardiac myoblasts in vitro. *J Mol Cell Cardiol* 1997;29:2585–2597.
- Bowes J, McDonald MC, Piper J, et al. Inhibitors of poly (ADP-ribose) synthetase protect rat cardiomyocytes against oxidant stress. *Cardiovasc Res* 1999;41:126–134.
- Grupp IL, Jackson TM, Hake P, et al. Protection against hypoxia-reoxygenation in the absence of poly (ADP-ribose) synthetase in isolated working hearts. *J Mol Cell Cardiol* 1999;31:297–303.
- Zingarelli B, Cuzzocrea S, Zsengeller Z, et al. Protection against myocardial ischemia and reperfusion injury by 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase. *Cardiovasc Res* 1997;36:205–215.
- Wayman N, McDonald MC, Thompson AS, et al. 5-aminoisoquinolinone, a potent inhibitor of poly (adenosine 5'-diphosphate ribose) polymerase, reduces myocardial infarct size. *Eur J Pharmacol* 2001;430:93–100.
- Bowes J, Ruetten H, Martorana PA, et al. Reduction of myocardial reperfusion injury by an inhibitor of poly (ADP-ribose) synthetase in the pig. *Eur J Pharmacol* 1998;359:143–150.
- Faro R, Toyoda Y, McCully JD, et al. Myocardial protection by PJ34, a novel potent poly (ADP-ribose) synthetase inhibitor. *Ann Thorac Surg* 2002;73:575–581.
- Uematsu M, Gaudette GR, Laurikka JO, et al. Adenosine-enhanced ischemic preconditioning decreases infarct in the regional ischemic sheep heart. *Ann Thorac Surg* 1998;66:382–387.
- Clermont G, Vergely C, Jazayeri S, et al. Systemic free radical activation is a major event involved in myocardial oxidative stress related to cardiopulmonary bypass. *Anesthesiology* 2002;96:80–87.
- Hayashi Y, Sawa Y, Ohtake S, et al. Peroxynitrite formation from human myocardium after ischemia-reperfusion during open heart operation. *Ann Thorac Surg* 2001;72:571–576.
- Paparella D, Yau TM, Young E. Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update. *Eur J Cardiothorac Surg* 2002;21:232–244.
- Ma XL, Weyrich AS, Lefer DJ, et al. Monoclonal antibody to L-selectin attenuates neutrophil accumulation and protects ischemic reperfused cat myocardium. *Circulation* 1993;88:649–658.
- Weyrich AS, Ma XY, Lefer DJ, et al. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J Clin Invest* 1993;91:2620–2629.
- Ma XL, Lefer DJ, Lefer AM, et al. Coronary endothelial and cardiac protective effects of a monoclonal antibody to intercellular adhesion molecule-1 in myocardial ischemia and reperfusion. *Circulation* 1992;86:937–946.
- Morse DS, Adams D, Magnani B. Platelet and neutrophil activation during cardiac surgical procedures: impact of cardiopulmonary bypass. *Ann Thorac Surg* 1998;65:691–695.
- Ilton MK, Langton PE, Taylor ML, et al. Differential expression of neutrophil adhesion molecules during coronary artery surgery with cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1999;118:930–937.
- Szabo C, Virag L, Cuzzocrea S, et al. Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly(ADP-ribose) synthase. *Proc Natl Acad Sci USA* 1998;95:3867–3872.
- Sawa Y, Taniguchi K, Kadoba K, et al. Leukocyte depletion attenuates reperfusion injury in patients with left ventricular hypertrophy. *Circulation* 1996;93:1640–1646.
- Zingarelli B, Salzman AL, Szabo 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.
- Khandoga A, Enders G, Biberthaler P, et al. Poly(ADP-ribose) polymerase triggers the microvascular mechanisms of hepatic ischemia-reperfusion injury. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G553–G560.
- Briaud SA, Ding ZM, Michael LH, et al. Leukocyte trafficking and myocardial reperfusion injury in ICAM-1/P-selectin-knockout mice. *Am J Physiol Heart Circ Physiol* 2001;280:H60–H67.
- Oliver FJ, Meissner-de Murcia J, Nacci C, et al. Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. *EMBO J* 1999;18:4446–4454.
- Garcia Soriano F, Virag L, Jagtap P, et al. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. *Nat Med* 2001;7:108–113.
- Komjati K, Jagtap PG, Baloglu E, et al. Poly(ADP-ribose) polymerase inhibition in stroke: establishment of the therapeutic window of intervention and delineation of its role in the pathogenesis of white matter damage. *FASEB J* 2002;16:A599.
- Szabo C, Bahrie S, Stumpf N, et al. Poly(ADP-ribose) polymerase inhibition reduces reperfusion injury after heart transplantation. *Circ Res* 2002;90:100–106.
- Pacher P, Liaudet L, Soriano FG, et al. The role of poly(ADP-ribose) polymerase activation in the development of myocardial and endothelial dysfunction in diabetes. *Diabetes* 2002;51:514–521.
- Pacher P, Liaudet L, Mabley J, et al. Pharmacologic inhibition of poly(adenosine diphosphate-ribose) polymerase may represent a novel therapeutic approach in chronic heart failure. *J Am Coll Cardiol* 2002;40:1006–1016.
- Goldfarb RD, Marton A, Szabo E, et al. Protective effect of a novel, potent inhibitor of poly(adenosine 5'-diphosphate-ribose) synthetase in a porcine model of severe bacterial sepsis. *Crit Care Med* 2002;30:974–980.
- Pacher P, Liaudet L, Bai P, et al. Activation of poly(ADP-ribose) polymerase contributes to development of doxorubicin-induced heart failure. *J Pharmacol Exp Ther* 2002;300:862–867.
- Baumgartner WA, Walinsky PL, Salazar JD, et al. Assessing the impact of cerebral injury after cardiac surgery: will determining the mechanism reduce this injury? *Ann Thorac Surg* 1999;67:1871–1873; discussion 1891–1894.
- Virag L, Szabo C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. *Pharmacol Rev* 2002;54:375–429.