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Short Communication

Role of poly(ADP-ribose) polymerase activation in endotoxin-induced cardiac collapse in rodents

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Abstract

Reactive oxygen and nitrogen species are overproduced in the cardiovascular system during circulatory shock. Oxidant-induced cell injury involves the activation of poly(ADP-ribose) polymerase (PARP). Using a dual approach of PARP-1 suppression, by genetic deletion or pharmacological inhibition with the new potent phenanthridinone PARP inhibitor PJ34 [the hydrochloride salt of *N*-(oxo-5,6-dihydrophenanthridin-2-yl)-*N*,*N*-dimethylacetamide], we studied whether the impaired cardiac function in endotoxic shock is dependent upon the PARP pathway. *Escherichia coli* endotoxin (lipopolysaccharide, LPS) at 55 mg/kg, i.p., induced a severe depression of the systolic and diastolic contractile function, tachycardia, and a reduction in mean arterial blood pressure in both rats and mice. Treatment with PJ34 significantly improved cardiac function and increased the survival of rodents. In addition, LPS-induced depression of left ventricular performance was significantly less pronounced in *PARP-1* knockout mice ($PARP^{-/-}$) as compared with their wild-type littermates ($PARP^{+/+}$). Thus, PARP activation in the cardiovascular system is an important contributory factor to the cardiac collapse and death associated with endotoxin shock.

Keywords: Poly(ADP-ribose) polymerase; Endotoxin; Shock; Cardiac collapse; Cardiac function

1. Introduction

PARP is an abundant nuclear enzyme of eukaryotic cells with multiple regulatory functions. When activated by DNA single-strand breaks, PARP initiates an energy-consuming cycle by transferring ADP ribose units from NAD^+ to nuclear proteins resulting in a rapid depletion of the intracellular NAD^+ and ATP pools; this, in turn, slows the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death [1]. Overactivation of PARP represents an important mechanism of tissue damage in various pathological conditions associated with increased oxidative stress including myocardial reperfusion injury [2,3], reperfusion injury after heart transplantation [4], drug-induced heart failure [5], stroke [6], autoimmune beta-cell destruction [7,8], cardiovascular dysfunction in diabetes [9–11], and circulatory shock [12–15].

Here we studied whether the impaired cardiac function in endotoxic shock is dependent upon the PARP pathway.

2. Materials and methods

The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23 revised 1996) and was performed with the approval of the local Institutional Animal Care and Use Committee.

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Abbreviations: PARP, poly(ADP-ribose) polymerase; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α; ICAM-1, intercellular adhesion molecule-1; IL, interleukin.

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2.1. Animals

Male $PARP^{+/+}$, $PARP^{-/-}$ mice and Wistar rats, weighing 25–30 g (mice) and 320–370 g (rats), were administered a single dose of *Escherichia coli* Serotype 0127:B8 LPS (endotoxin; 55 mg/kg, i.p.; Sigma/Aldrich, Lot71K4080) and used for functional measurements 16–20 hr later. Treatment with the PARP inhibitor PJ34 [the hydrochloride salt of *N*-(oxo-5,6-dihydro-phenanthridin-2-yl)-*N*,*N*-dimethylacetamide; 10 and 20 mg/kg, i.p.] started 1 hr before the endotoxin injection and was repeated 10 hr later in both mice and rats. A similar dosing regimen with PJ34 has been shown previously to be sufficient to block vascular and cardiac PARP activation in rats and mice [4,5,9–11,16,17].

2.2. Hemodynamic measurements in rats and mice

Sixteen to twenty hours after LPS administration, analysis of left ventricular performance was measured in mice anesthetized with i.p. injections of ketamine (80 mg/kg) and xylazine (10 mg/kg) and in rats anesthetized with i.p. injections of thiopentone sodium (60 mg/kg) as described previously [5,9,18]. Briefly, the animals were placed on controlled heating pads, and core temperature measured via a rectal probe was maintained at 36–38°. A microtip catheter transducer (SPR-671 in mice and SPR-524 in rats; Millar Instruments) was inserted into the right carotid artery and advanced into the left ventricle under pressure control. After stabilization for 15–20 min, the pressure signal was recorded continuously using a MacLab A/D converter (AD Instruments), and stored and displayed on an Apple Macintosh personal computer. The heart rate and the left ventricular systolic and end-diastolic pressures 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 for the measurement of arterial blood pressure. After the hemodynamic measurements were made, the animals were killed.

2.3. Statistical analysis

Results are reported as means ± SEM. Statistical significance between two measurements was determined by the two-tailed unpaired Student's *t*-test, and among groups it was determined by analysis of variance with Bonferroni's correction. In the survival experiments, the survival curves

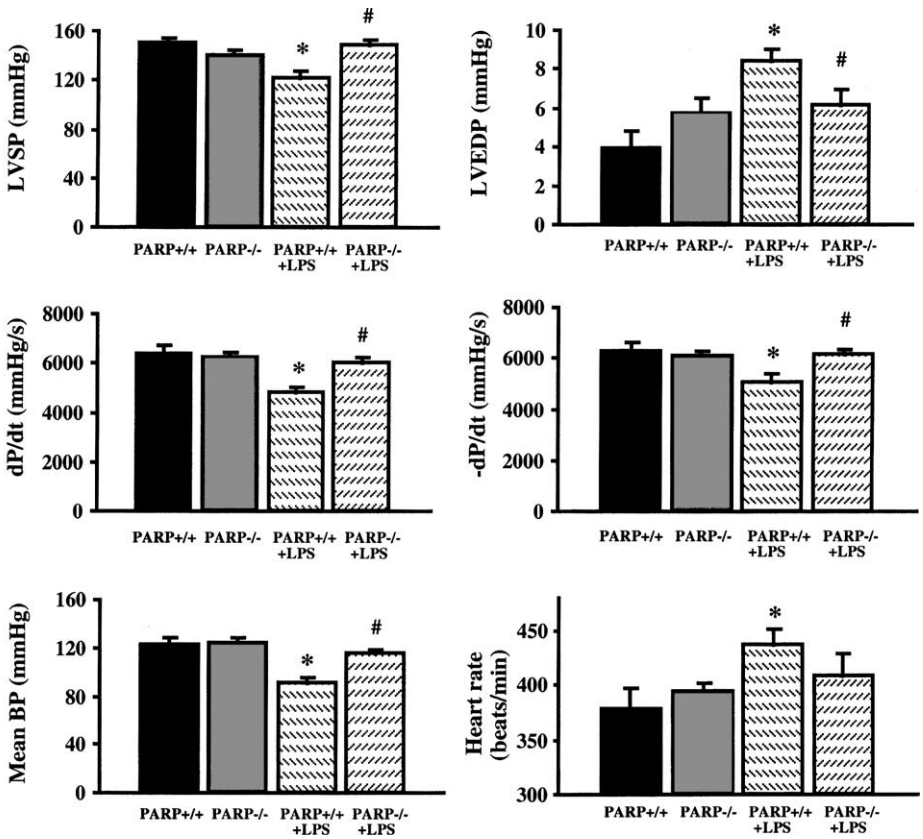

Fig. 1. Effect of genetic deletion of PARP-1 on endotoxin (LPS)-induced cardiac dysfunction in mice. Effect of a single dose of LPS (55 mg/kg, i.p.) on left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular −dP/dt, mean blood pressure (mean BP), and heart rate in $PARP^{+/+}$ and $PARP^{-/-}$ mice. Hemodynamic parameters were measured 16–20 hr after LPS administration. Results are means ± SEM of 7–9 experiments in each group. Key: (*) $P < 0.05$ vs $PARP^{+/+}$; and (#) $P < 0.05$ vs $PARP^{+/+}$ +LPS.

of the different groups were compared using the log-rank test. Probability values of $P < 0.05$ were considered significant.

2.4. Reagents

All reagents were obtained from Sigma/Aldrich, unless indicated otherwise. PJ34, a potent, novel, water-soluble phenantridinone derivative PARP inhibitor, was synthesized as described [10]. In the cell-free PARP assay, using NAD^+ and purified PARP-1 enzyme, PJ34 inhibited PARP activity in a concentration-dependent manner, with an EC_{50} of 20 nM. The EC_{50} of a prototypical PARP inhibitor, 3-aminobenzamide, was 200 μM. Peroxynitrite- and hydrogen peroxide-induced oxidation of dihydrorhodamine-123 was unaffected by PJ34, in the concentration range of 1 μM to 10 mM, indicating that the compound does not act as an antioxidant. Details of the synthesis and pharmacological characterization of PJ34 were published previously [10].

3. Results

3.1. Cardiac function

3.1.1. Effects of genetic deletion and pharmacological inhibition of PARP-1 on LPS-induced cardiac dysfunction in mice

In $PARP^{+/+}$ mice treated with LPS (55 mg/kg, i.p.), mean blood pressure, left ventricular systolic pressure, +dP/dt, and −dP/dt were decreased significantly, whereas heart rate and left ventricular end-diastolic pressure increased (Figs. 1 and 2). In contrast, $PARP^{-/-}$ mice treated with LPS showed normal left ventricular performance (Fig. 1). There was no significant difference in the left ventricular function between $PARP^{+/+}$ and $PARP^{-/-}$ mice in the absence of LPS treatment (Fig. 1).

PJ34 treatment, similarly to genetic deletion of the PARP-1 enzyme, was associated with significantly improved cardiac performance and mean blood pressure in the endotoxin-treated mice (Fig. 2). The PARP inhibitor

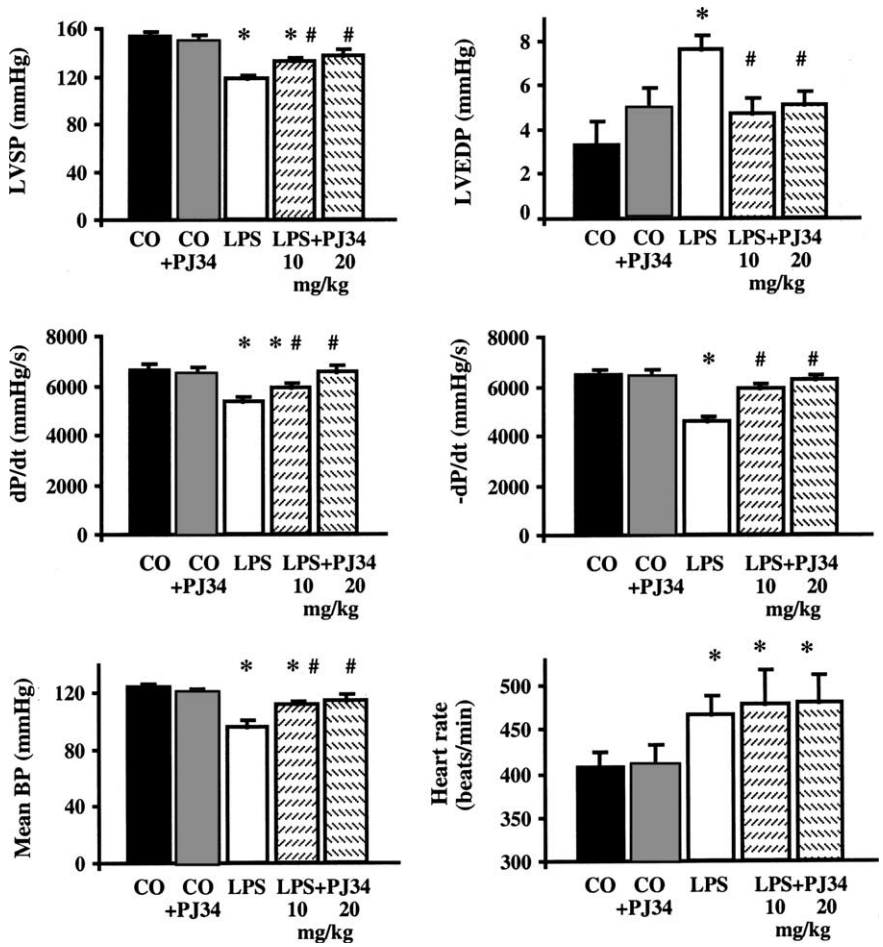

Fig. 2. Effect of pharmacological inhibition of PARP on endotoxin (LPS)-induced cardiac dysfunction in mice. Effect of LPS and PJ34 on left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular −dP/dt, mean blood pressure (mean BP), and heart rate in $PARP^{+/+}$ mice. CO, control; LPS, endotoxin treated (55 mg/kg, i.p.); CO+PJ34, control treated with PJ34 (2 × 20 mg/kg, i.p.); LPS+PJ34, endotoxin (55 mg/kg, i.p.) and PJ34 (2 × 10 and 2 × 20 mg/kg i.p.) treated. Hemodynamic parameters were measured 16–20 hr after endotoxin administration. Results are means ± SEM of 6–8 experiments in each group. Key: (*) $P < 0.05$ vs CO; and (#) $P < 0.05$ vs LPS.

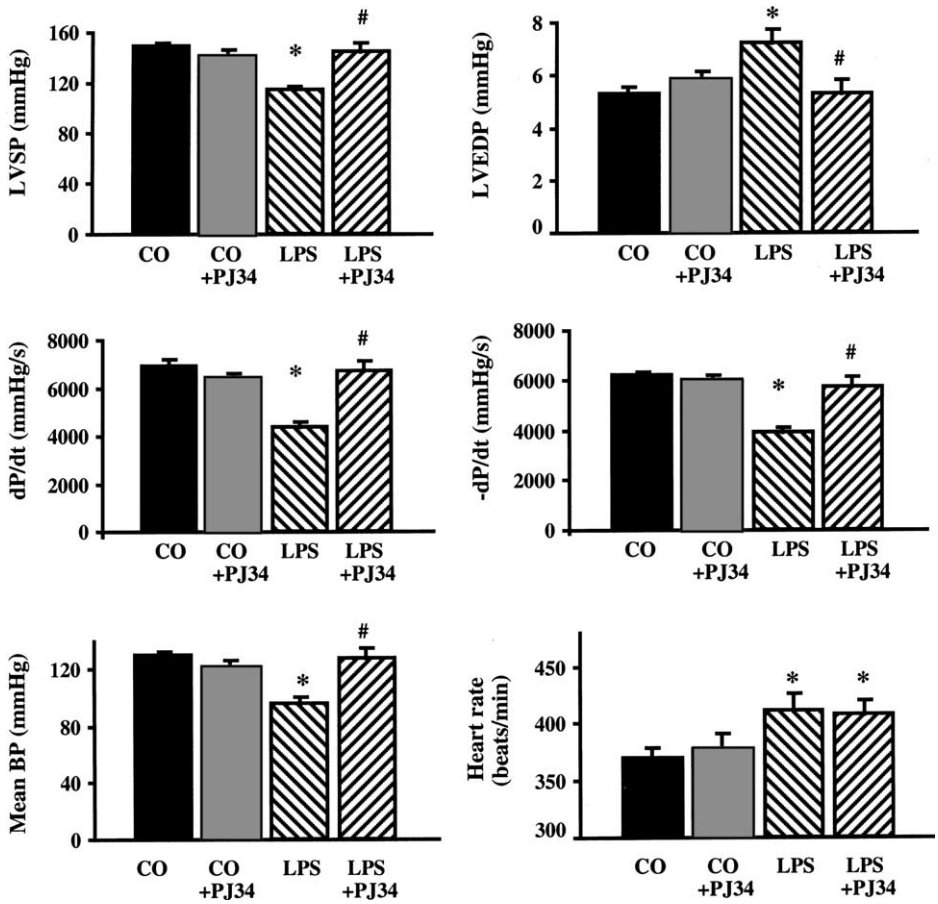

Fig. 3. Effect of pharmacological inhibition of PARP on endotoxin (LPS)-induced cardiac dysfunction in rats. Effect of LPS and PJ34 on left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular +dP/dt, left ventricular −dP/dt, mean blood pressure (mean BP), and heart rate in Wistar rats. CO, control; LPS, endotoxin treated (55 mg/kg, i.p.); CO+PJ34, control treated with PJ34 (2 × 20 mg/kg, i.p.); LPS+PJ34, endotoxin (55 mg/kg, i.p.) and PJ34 (2 × 20 mg/kg, i.p.) treated. Hemodynamic parameters were measured 16–20 hr after endotoxin administration. Results are means ± SEM of 7–10 experiments in each group. Key: (*) $P < 0.05$ vs CO; and (#) $P < 0.05$ vs LPS.

had no significant effects on hemodynamic parameters in the control mice (Fig. 2).

3.1.2. *Effects of PJ34 on the LPS-induced cardiac dysfunction in Wistar rats*

LPS induced a significant increase in heart rate and left ventricular end-diastolic pressure and a decrease in mean blood pressure, left ventricular systolic pressure, +dP/dt, and −dP/dt in Wistar rats (Fig. 3). Treatment with PJ34 normalized the LPS-induced changes in left ventricular systolic pressure, mean blood pressure, systolic +dP/dt, diastolic −dP/dt, and left ventricular end-diastolic pressure but not in the heart rate (Fig. 3). The PARP inhibitor had no significant effects on hemodynamic parameters in the control rats (Fig. 3).

3.2. *Survival of animals*

The results of the survival experiments are shown in Fig. 4. Treatment with PJ34 significantly decreased the LPS-induced mortality in both mice (Fig. 4A) and rats (Fig. 4B). All $PARP^{-/-}$ mice were alive at the time when functional measurements were performed (16–20 hr after LPS administration), and since previous studies have already documented an improved survival in these animals to endotoxin [13,14], mortality was not followed afterwards.

4. Discussion

The present study demonstrated severe depression of left ventricular function involving both systolic pressure development and relaxation 16–20 hr after the administration of LPS in well-established mouse and rat models of endotoxin shock (Figs. 1–3). Importantly, the results presented here document, for the first time, that in endotoxin shock, the activation of PARP in the myocardium may contribute to the impaired cardiac function, since $PARP^{-/-}$ mice were more resistant to the cardiodepressant effects of LPS than $PARP^{+/+}$ mice (Fig. 1), and the pharmacological inhibition of PARP with PJ34 attenuated the endotoxin-induced cardiac dysfunction in both mice and rats (Figs. 2 and 3). Furthermore, PJ34 treatment significantly increased the survival of the mice and rats treated with LPS (Fig. 4).

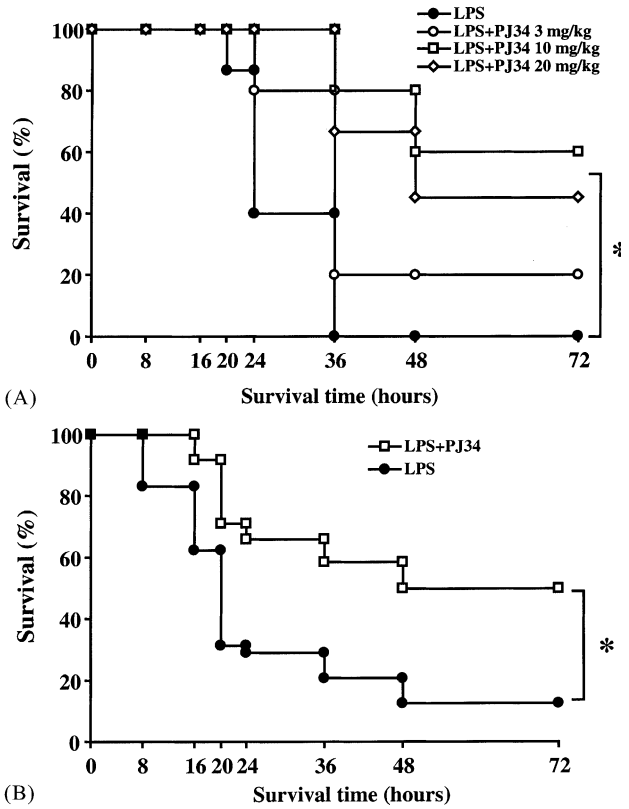

Fig. 4. Effect of pharmacological inhibition of PARP on survival of mice (A) and rats (B) treated with endotoxin (LPS). Panel A: Treatment with PJ34 [2 × 3 (N = 10), 10 (N = 10), or 20 (N = 15) mg/kg/day] significantly decreased LPS (a single dose of 55 mg/kg, i.p.)-induced mortality. In the LPS group, N = 15. Key: (*) $P < 0.05$, log-rank test. Panel B. Treatment with PJ34 (2 × 20 mg/kg/day; N = 24) significantly decreased the LPS (55 mg/kg, i.p.)-induced mortality. In the LPS group, N = 48. Key: (*) $P < 0.05$, log-rank test.

These results are in agreement with previous data demonstrating the improved survival of mice lacking PARP-1 enzyme in endotoxic and septic shock models [13,14,19]. We have shown previously that the dose regimen of PJ34 used in the current study effectively inhibits PARP activation in different tissues including heart in various pathophysiological conditions [4,5,9–11,16,17,20–22].

As far as the mechanisms of the protective effects of PARP inhibitors are concerned, two main pathways have been identified: PARP—in its basal, constitutive state—can act as a co-inducer of pro-inflammatory gene expression in shock [1,13], and PARP activation can mediate inflammatory cell dysfunction and ultimately cell necrosis [1]. These two pathways may be interrelated in shock, because by reducing the production of inflammatory chemokines and cytokines PARP inhibition reduces the amount of tissue-infiltrating mononuclear cells. This results in the production of less genotoxic oxidants and free radicals, thereby attenuating the degree of DNA single-strand breakage and PARP activation, thus preventing energy depletion leading to cell dysfunction and/or necrosis. The beneficial effects of PARP deficiency or PARP inhibitors on functional contractile parameters and on high-energy phosphates after global ischemia/reperfusion of the heart or heart transplantation [4,23,24] have been reported.

The fact that PARP can regulate the expression of inflammatory mediators has been the subject of much research interest. Suppression of the expression of iNOS, TNF-α, and ICAM-1 has been reported in PARP-deficient mice and in the presence of pharmacological inhibition of PARP [3,25]. Whether it is the catalytic activity of PARP or the actual presence of PARP that is important in the regulation of inflammatory gene expression is a controversial subject. For example, in endotoxic shock, Ménissier-de Murcia and colleagues [13] found that PARP deficiency but not the pharmacological inhibition of PARP was able to suppress the activation of NF-κB. Similarly, in endothelial cells exposed to high glucose to mimic diabetic vascular complications, we observed that PARP-1 deficiency but not PARP inhibition with PJ34 suppresses the activation of NF-κB [10]. On the other hand, both PARP inhibition and deficiency were found to inhibit the expression of iNOS and the expression of ICAM-1 in various experimental systems [3,26]. The activation of the HIV-LTR promoter and an NF-κB-dependent artificial promoter has been shown to be reduced drastically in PARP-deficient cells. Furthermore, NF-κB-dependent gene activation could be restored by the expression of PARP in PARP-deficient cells. In one series of studies, it appeared that NF-κB and PARP formed a stable immunoprecipitable nuclear complex, and this interaction does not need DNA binding [27]. Hassa and colleagues [28] demonstrated that a PARP-1 mutant lacking the enzymatic and DNA binding activity interacted comparably to the wild-type PARP-1 with p65 or p50, concluding that the enzymatic activity of the enzyme is not essential for its interaction with NF-κB. In contrast, Chang and Alvarez-Gonzalez [29] concluded that NF-κB-p50 DNA-binding was dependent upon the presence of NAD; DNA-binding by NF-κB-p50 was not efficient in the absence of NAD, and was blocked in the presence of pharmacological inhibitors of PARP, allowing the conclusion that NF-κB-p50 DNA-binding is protein-poly(ADP-ribosyl)ation dependent. It appears that the relative role of the presence of PARP versus its catalytic activity in the regulation of NF-κB activation may depend upon the experimental system used, the stimulus of induction, and possibly the cell type involved. Our recent work, both *in vitro* and *in vivo*, demonstrates that the regulation of the expression of various chemokines (MIP-1α, MIP-2) by PARP-1 is dependent upon the catalytic activity of the enzyme [30]. A recent study by Ha *et al.* [31] has clarified some of the issues related to the role of PARP (catalytic activity and physical presence) in the regulation of pro-inflammatory mediator production. It was demonstrated in immunostimulated glial cells that a whole host of transcription factors, including NF-κB, AP-1, SP-1, Oct-1, YY-1, and Stat-1 were down-regulated in the absence of PARP, and a host of pro-inflammatory mediators were reduced. Parallel experiments with an isoquinolinone PARP inhibitor

demonstrated that the activation of NF-κB does not require catalytic activity of PARP, but the production of some of the pro-inflammatory mediators (such as IL-1β and iNOS-derived NO) is dependent upon the catalytic activity of the enzyme [31]. Clearly, further work is required for the exact clarification of the conditions where the presence of PARP versus its catalytic activity regulates the activation of NF-κB and the expression of inflammatory genes, and the various biological responses. Based on the current results showing comparable effects of PARP deficiency and PARP inhibition against LPS-induced cardiac dysfunction, we hypothesize that the myocardial protection observed here is likely related to the catalytic activity of PARP.

In conclusion, our results demonstrate that the reactive oxygen/nitrogen species–PARP pathway also plays a pathogenetic role in the development of endotoxin-induced cardiac dysfunction. Further work is required to clarify whether PARP inhibition may exert beneficial effects against cardiac collapse associated with shock in humans.

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References

[1] Virag L, Szabó C. The therapeutic potential polymerase inhibitors. Pharmacol Rev 2002;54:375–429.
[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 USA 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, Bährle S, Stumpf N, Sonnenberg K, Szabó E, Pacher P, Csont T, Schulz R, Denger TJ, Liaudet L, Jagtap PG, Southan GJ, Vahl CF, Hagl S, Szabó C. Poly(ADP-ribose) polymerase inhibition reduces reperfusion injury after heart transplantation. Circ Res 2002;90:100–6.
[5] Pacher P, Liaudet L, Bai P, Virag L, Mabley JG, Haskó G, Szabó C. Activation of poly(ADP-ribose) polymerase contributes to the development of doxorubicin-induced heart failure. J Pharmacol Exp Ther 2002;300:862–7.
[6] Eliasson MJ, Sampei K, Mandir AS, Hurn PD, Traystman RJ, Bao J, Pieper A, Wang ZQ, Dawson TM, Snyder SH, Dawson VL. Poly(-ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. Nat Med 1997;3:1089–95.
[7] Burkart V, Wang Z-Q, Radons J, Heller B, Herceg Z, Stingl L, Wagner EF, Kolb H. 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.
[8] Pieper AA, Brat DJ, Krug DK, Watkins CC, Gupta A, Blackshaw S, Verma A, Wang ZQ, Snyder SH. Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. Proc Natl Acad Sci USA 1999;96:3059–64.
[9] Pacher P, Liaudet L, Soriano FG, Mabley JG, Szabó E, Szabó C. The role of poly(ADP-ribose) polymerase in the development of cardiovascular dysfunction in diabetes mellitus. Diabetes 2002;51:514–21.
[10] Soriano FG, Virág L, Jagtap P, Szabó E, Mabley JG, Liaudet L, Marton A, Hoyt DG, Murthy KGK, Salzman AL, Southan GJ, Szabó C. Diabetic endothelial dysfunction: the role of poly(ADP-ribose) polymerase activation. Nat Med 2001;7:108–13.
[11] 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.
[12] 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;100:723–5.
[13] Oliver FJ, Ménissier-de Murcia J, Nacci C, Decker P, Andriantsitohaina R, Muller S, de la Rubia G, Stoclet JC, de Murcia G. Resistance to endotoxic shock as a consequence of defective NF-κB activation in poly(ADP-ribose) polymerase-1 deficient mice. EMBO J 1999;18:4446–54.
[14] Kuhnle S, Nicotera P, Wendel A, Leist M. Prevention of endotoxin-induced lethality, but not of liver apoptosis in poly(ADP-ribose) polymerase-deficient mice. Biochem Biophys Res Commun 1999;263:433–8.
[15] Liaudet L, Soriano FG, Szabó E, Virág L, Mabley JG, Salzman AL, Szabó C. Protection against hemorrhagic shock in mice genetically deficient in poly-(ADP-ribose) polymerase. Proc Natl Acad Sci USA 2000;97:10203–8.
[16] Goldfarb RD, Marton A, Szabó E, Virág L, Salzman AL, Glock D, Akhter I, McCarthy R, Parrillo JE, Szabó C. 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;30:974–80.
[17] Faro R, Toyoda Y, McCully JD, Jagtap P, Virág L, Bianchi C, Levitsky S, Szabó C, Sellke FW. Myocardial protection by PJ34, a novel potent poly(ADP-ribose) synthetase inhibitor. Ann Thorac Surg 2002;73:575–81.
[18] Liaudet L, Szabó E, Timashpolsky L, Virág L, Cziráki 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] Soriano FG, Liaudet L, Szabó E, Virág L, Mabley JG, Pacher P, Szabó C. Resistance to acute septic peritonitis in poly(ADP-ribose) polymerase-1-deficient mice. Shock 2002;17:286–92.
[20] Jagtap P, Soriano FG, Virág L, Liaudet L, Mabley J, Szabó E, Haskó G, Marton A, Lorigados CB, Gallyas Jr F, Sümegi B, Hoyt DG, Baloglu E, VanDuzer J, Salzman AL, Southan GJ, Szabó C. Novel phenanthridinone inhibitors of poly(adenosine 5(-diphosphate-ribose) synthetase: potent cytoprotective and anti-shock agents. Crit Care Med 2002;30:1071–82.
[21] Mabley JG, Jagtap P, Perretti M, Getting SJ, Salzman AL, Virág L, Szabó E, Soriano FG, Liaudet L, Abdekarim GF, Haskó G, Marton A, Southan GJ, Szabó C. Anti-inflammatory effects of a novel potent inhibitor of poly(ADP-ribose) polymerase. Inflamm Res 2001;50:561–9.
[22] Liaudet L, Pacher P, Mabley JG, Virág L, Soriano FG, Haskó G, Szabó C. Activation of poly(ADP-ribose) polymerase-1 is a central mechanism of lipopolysaccharide-induced acute lung inflammation. Am J Respir Crit Care Med 2002;165:372–7.
[23] 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.
[24] 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.

[25] Zingarelli B, Szabó C, Salzman AL. Blockade of poly(ADP-ribose) synthetase inhibits neutrophil recruitment, oxidant generation, and mucosal injury in murine colitis. Gastroenterology 1999;116: 335–45.

[26] Szabó C, Virág L, Cuzzocrea S, Scott GS, Hake P, O'Connor MP, Zingarelli B, Salzman A, Kun E. Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly(ADP-ribose) synthase. Proc Natl Acad Sci USA 1998;95: 3867–72.

[27] Hassa PO, Hottiger MO. A role of poly(ADP-ribose) polymerase in NF-κB transcriptional activation. Biol Chem 1999;380:953–9.

[28] Hassa PO, Covic M, Hasan S, Imhof R, Hottiger MO. The enzymatic and DNA binding activity of PARP-1 are not required for NF-κB coactivator function. J Biol Chem 2001;276:45588–97.

[29] Chang W-J, Alvarez-Gonzalez R. The sequence-specific DNA binding of NF-κB is reversibly regulated by the automodification reaction of poly(ADP-ribose) polymerase 1. J Biol Chem 2001;276:47664–70.

[30] Haskó G, Mabley JG, Németh ZH, Pacher P, Deitch EA, Szabó C. Poly(ADP-ribose) polymerase is a regulator of chemokine production: relevance for the pathogenesis of shock and inflammation. Mol Med 2002;8:283–9.

[31] Ha HC, Hester LD, Snyder SH. Poly(ADP-ribose) polymerase-1 dependence of stress-induced transcription factors and associated gene expression in glia. Proc Natl Acad Sci USA 2002;99:3270–5.