The Role of Poly(ADP-Ribose) Polymerase Activation in the Development of Myocardial and Endothelial Dysfunction in Diabetes

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Patients with diabetes exhibit a high incidence of diabetic cardiomyopathy and vascular complications, which underlie the development of retinopathy, nephropathy, and neuropathy and increase the risk of hypertension, stroke, and myocardial infarction. There is emerging evidence that the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP) importantly contributes to the development of endothelial dysfunction in a streptozotocin-induced model of diabetes. We investigated the role of PARP activation in the pathogenesis of cardiac dysfunction in streptozotocin-induced and genetic (nonobese diabetic) models of diabetes in rats and mice. Development of diabetes was accompanied by hyperglycemia, cardiac PARP activation, a selective loss of endothelium-dependent vasodilation in the thoracic aorta, and an early diastolic dysfunction of the heart. Treatment with a novel potent phenanthridinone-based PARP inhibitor, PJ34, starting 1 week after the onset of diabetes, restored normal vascular responsiveness and significantly improved cardiac dysfunction, despite the persistence of severe hyperglycemia. The beneficial effects of PARP inhibition persisted even after several weeks of discontinuation of the treatment. Thus, PARP activation plays a central role in the pathogenesis of diabetic cardiovascular (cardiac as well as endothelial) dysfunction. PARP inhibitors may exert beneficial effects against the development of cardiovascular complications in diabetes. Diabetes 51:514–521, 2002

RESEARCH DESIGN AND METHODS
The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by U.S. National Institutes of Health (NIH publication no. 85-23, revised 1985) and was performed with the approval of the local Institutional Animal Care and Use Committee.

Experimental models of diabetes

Genetic model of diabetes in mice. A total of 72 adult female NOD mice weighing (20–30 g) were used for studies. Mice developing severe hyperglycemia (>350 mg/dl) and glucosuria for two consecutive days were selected and considered diabetic (n = 40). NOD mice with normal (<200 mg/dl) blood and urine glucose concentrations were used as controls (n = 32). PARP was inhibited by PJ34 (10 mg/kg oral gavage, once daily, starting 1 week after the development of diabetes). This dose regimen was found to effectively inhibit vascular PARP activation in previous studies (9,10). Diabetic and control mice were killed after 4 weeks of treatment with PJ34 or vehicle.

STZ-induced model of diabetes in rats. Diabetes was induced in male Wistar rats (n = 50) weighing 240–260 g by use of a single injection of STZ (50 mg/kg i.v.) dissolved in the citrate-saline solution (pH 4.5) into the tail vein. Control animals (n = 30) were injected with the vehicle alone. Diabetes was confirmed by the presence of hyperglycemia (>200 mg/dl). One week after the injection of STZ, control and diabetic rats received either vehicle or the PARP

ADP ribose units from NAD+ to nuclear proteins. This process results in rapid depletion of the intracellular NAD+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death (1–7). Overactivation of PARP represents an important mechanism of tissue damage in various pathological conditions associated with oxidant stress, including myocardial reperfusion injury (4), stroke (3), shock (7,8), and autoimmune β-cell destruction (5,6). Recently, we reported that the activation of PARP importantly contributes to the development of endothelial dysfunction in a streptozotocin (STZ)-induced model of diabetes in mice (9,10).

Cardiovascular complications are the most common cause of morbidity and mortality in diabetic patients. The presence of myocardial dysfunction independent of coronary artery disease in diabetes, known as “diabetic cardiomyopathy,” has been well documented in both humans and animals (11–15). Diabetic cardiomyopathy is characterized by an early diastolic dysfunction and a late systolic one, with intracellular retention of calcium and sodium and loss of potassium. The mechanism of diastolic dysfunction remains unknown, but it does not appear to be due to changes in blood pressure, microvascular complications, or elevated circulating glycated hemoglobin levels (15,16). We tested whether the impairment of cardiac function in diabetes is dependent upon the activation of the PARP pathway within the heart.
inhibitor PJ34 (10 mg/kg oral gavage, once daily). Rats and age-matched controls were killed after 4 weeks of treatment.

In a separate set of experiments, diabetic (n = 12) and control rats (n = 12) were treated with PJ34 (10 mg/kg oral gavage, once daily) for 6 weeks, and then treatment was stopped and animals were followed for a subsequent period of 3 weeks. At 10 weeks, animals were subjected to cardiovascular measurements.

Blood glucose was measured using a one-touch blood glucose meter (LifeScan, Milpitas, CA). Total glycated hemoglobin was measured using a commercially available kit (Sigma, St. Louis, MO). Pancreatic insulin content in NOD mice was measured in the supernatant of pancreatic homogenates using an enzyme-linked immunosorbent assay as previously described (9).

Measurement of vascular reactivity in isolated aortic rings of NOD mice. The method was described previously in detail (9). Briefly, the thoracic aorta was cleared from perivascular fat and cut into 0.5–1.0 mm width rings using operation microscope, mounted in organ baths filled with warmed (37°C) and oxygenated (95% O2/5% CO2) Krebs’ solution (CaCl2 1.6 mmol/l; MgSO4 1.17 mmol/l; EDTA 0.026 mmol/l; NaCl 130 mmol/l; NaHCO3 14.9 mmol/l; KC1 4.7 mmol/l; KH2PO4 1.18 mmol/l; and glucose 11 mmol/l). Isometric tension was measured with isometric transducers (Kent Scientific Corporation, Litchfield, CT), digitalized using a MacLab A/D converter and stored and displayed on an Apple Macintosh personal computer. The heart rate, the left ventricular end-diastolic pressure (LVEDP), the thoracic systolic pressure (SAP), the rate of LV shortening (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 counted.

General characteristics of the animals. Progression of diabetes in NOD mice and after STZ injection in rats resulted in a decrease in the growth of the diabetic animals (Fig. 1). Body weight decreased by 16 and 35% by 5 weeks, and heart weight decreased by 12 and 26% by 5 weeks, thereby increasing the heart weight-to-body weight ratio by 5 and 13% in mice and rats, respectively. Diabetic mice and rats also exhibited increased serum concentrations of glucose and glycated hemoglobin (Fig. 1). Treatment of diabetic and control rats and mice with the PARP inhibitor PJ34 did not significantly influence body and heart weights or plasma levels of glucose and glycated hemoglobin. Pancreas insulin content (nanogram of insulin per milligram of pancreatic protein) was 40.3 ± 4.1 (n = 10), 45.9 ± 2.5 (n = 7), 1.9 ± 0.4 (n = 14), and 1.8 ± 0.3 (n = 9) in NOD control mice, NOD control mice treated with PJ34 for 4 weeks, NOD diabetic mice, and NOD diabetic mice treated with PJ34 for 4 weeks, respectively.

RESULTS

Ventricular function in NOD mice. In untreated diabetic mice, heart rate, mean blood pressure, left ventricular systolic pressure, +dP/dt, and −dP/dt were significantly decreased, whereas left ventricular end-diastolic pressure increased. Treatment with PJ34 prevented the depression in left ventricular systolic pressure, +dP/dt, −dP/dt and the elevation of the left ventricular end-diastolic pressure (Fig. 2). PJ34 slightly improved the decreased heart rate but did not reverse the decrease in mean arterial blood pressure (Fig. 2) in diabetic mice. The PARP inhibitor treatment had no significant effects on hemodynamic parameters in control mice (Fig. 2).

Ventricular function in rats. Similar to mice, diabetes in rats was characterized by an increase in left ventricular end-diastolic pressure and a decrease in heart rate, mean
blood pressure, left ventricular systolic pressure, +dP/dt, and –dP/dt (Fig. 3). PJ34 treatment significantly improved the depression in left ventricular systolic pressure and diastolic –dP/dt. Moreover, the elevation of the left ventricular end-diastolic pressure and the decrease of systolic +dP/dt was completely prevented by the PARP inhibitor treatment. The PJ34 treatment also improved the decreased heart rate and mean blood pressure in diabetic rats. Similar to the findings in mice, PJ34 treatment in normal (nondiabetic) rats had no significant effects on hemodynamic parameters (Fig. 3).

In a separate set of experiments, diabetic rats (n = 12) were treated with PJ34 for 6 weeks followed by the discontinuation of the treatment for an additional 3-week period. The improved cardiac function in these rats was still maintained at 3 weeks after discontinuation of the treatment, when compared with the matched 10-week diabetic animals (Fig. 4).

**Evidence for PARP activation in the diabetic hearts in vivo.** As shown in Fig. 5, a marked degree of PARP activation was observed in the hearts isolated from 5- and 10-week-old diabetic rats. The staining was mainly localized in the nuclei of cardiac myocytes.

Similarly, immunohistochemical evidence of PARP activation was found in the hearts of the diabetic NOD mice (Fig. 6). In both rats and mice, the PARP activation was markedly attenuated by treatment with PJ34 (Figs. 5–6). The percentage of PARP-positive nuclei of myocytes was 1.7 ± 0.7% (n = 5) and 1.1 ± 0.3% (n = 5) in control nondiabetic rats and NOD mice, respectively. Five weeks of diabetes increased the fraction of PARP-positive nuclei to 23.6 ± 6% (n = 5, P < 0.01) and 21.6 ± 3.6% (n = 5, P < 0.01) in rats and NOD mice, respectively. Treatment with PJ34 (for 4 weeks) markedly decreased the percentage of PARP-positive nuclei to 4.3 ± 0.7% (n = 5, P < 0.01) and 3.3 ± 0.7% (n = 5, P < 0.01) in both rats and mice, respectively. At 10 weeks, in the hearts of the diabetic rats, the percentage of PARP-positive nuclei was 26.5 ± 5.5% (n = 5). Treatment with PJ34 (for 6 weeks) of diabetic rats even after discontinuation for 3 weeks reduced the percentage of PARP-positive nuclei to 7.1 ± 1.9% (n = 5, P < 0.01).

**Diabetes induces a PARP-dependent endothelial dysfunction in NOD mice.** Ex vivo experiments demonstrated the loss of endothelial function, as measured by the relaxant responsiveness of precontracted vascular rings to the endothelium-dependent vasodilator, nitric oxide–liberating hormone acetylcholine (Fig. 7). Inhibition of PARP activation was achieved by chronic treatment with the potent, water-soluble phenantridinone derivative PARP inhibitor PJ34, starting at 1 week after the onset of diabetes. This treatment restored normal vascular function (Fig. 7). The endothelium-independent relaxant response to sodium nitroprusside was unchanged (Fig. 7), indicating that the ability of the smooth muscle to relax to nitric oxide was not impaired in diabetes. The contractile
responsiveness of the thoracic aorta to norepinephrine was unchanged in the diabetic NOD mice. PJ34 treatment had no significant effects on contractile or endothelium-dependent and -independent relaxant responses in control animals (Fig. 7).

**DISCUSSION**

Diabetic cardiomyopathy is characterized by complex changes in the mechanical, biochemical, structural, and electrical properties of the heart, which may be responsible for the development of an early diastolic dysfunction and increased incidence of cardiac arrhythmias in diabetic patients. Despite the expanding knowledge obtained from different models of diabetes, the mechanism of diabetic cardiac diastolic dysfunction remains elusive (rev. in 14,15,18–20).

The present study demonstrates that both genetic and STZ-induced diabetes in NOD mice and rats were associated with a marked depression of left ventricular function involving both systolic pressure development and relaxation. These results are in agreement with earlier reports showing depressed cardiac performance in different mouse (21,22) and rat (13,23–27) models of diabetes. Importantly, the results presented here document for the first time that in both genetic and STZ-induced models of type 1 insulin-dependent diabetes, the impaired cardiac function is associated with an activation of PARP in the myocardium. Furthermore, we now show that the diabetic cardiac dysfunction can be reversed by pharmacological inhibition of PARP.

Clinical and experimental investigations suggested that increased sympathetic activity, activated cardiac renin-angiotensin system, myocardial ischemia/functi

**FIG. 3.** Reversal of STZ-evoked diabetes induced cardiac dysfunction by pharmacological inhibition of PARP in rats. Effect of diabetes (5 weeks) and PJ34 (4 weeks) 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 rats. C, control; D, diabetic (for 5 weeks); C+PJ34, control treated with PJ34 (for 4 weeks); D+PJ34, diabetic treated with PJ34 (treatment was started after 1 week of established diabetes for further 4 weeks). Data are means ± SE. *P < 0.05 vs. C; #P < 0.05 vs. D.

**FIG. 4.** Preservation of beneficial effects of pharmacological inhibition of PARP on cardiac dysfunction in STZ-induced diabetic rats after discontinuation of the treatment for 3 weeks. Effect of diabetes (10 weeks) 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 rats. C, control; D, diabetic; C+PJ34, control treated with PJ34 (treatment was started after 1 week of established diabetes for 6 weeks followed by discontinuation for 3 weeks); D+PJ34, diabetic rat treated with PJ34 (for 6 weeks). Data are means ± SE. *P < 0.05 vs. C; #P < 0.05 vs. D.
breakage is the obligatory trigger of PARP activation (1,2,7,34), which in turn may result in rapid depletion of the intracellular NAD⁺ and ATP pools, thus slowing the rate of glycolysis and mitochondrial respiration, which eventually leads to cellular dysfunction and death. The importance of the PARP pathway is well documented in various models of myocardial ischemia-reperfusion injury (another condition in which oxidative stress plays a key pathogenetic role) (4,35–42). Based on the results of the current study, we conclude that the “reactive oxygen/nitrogen species–DNA injury–PARP activation” pathway also plays a pathogenetic role in the development of diabetic cardiomyopathy.

In recent studies (9,10) we have demonstrated that in vitro and in vivo endothelial cell dysfunction in response to high glucose is associated with increased poly(ADP-ribose)ylation. Furthermore, endothelial function is maintained in vascular rings of animals in which PARP-1 is inactivated or PARP is pharmacologically inhibited. In the present study, we extended these findings by showing that the genetically diabetic NOD mice also develop severe endothelial dysfunction, which can be reversed by PARP inhibitor treatment. These findings further strengthen the view that the activation of PARP is an important factor in the pathogenesis of endothelial dysfunction in diabetes. We have also measured cellular nucleoside levels in endothelial cells exposed to high glucose, as well as in diabetic vascular rings, in the presence or absence of PJ34 treatment (9,10). It was observed that diabetes in vivo (10) and high glucose incubation in vitro (9) induces a severe metabolic suppression of the endothelial cells and of the blood vessels, characterized by NAD, NADPH, and ATP depletion, effects that could be partially restored by pharmacological inhibition of PARP (9,10). If similar mechanisms are operative in the diabetic hearts, it is conceivable that—in addition to a variety of other mechanisms—a direct energetic deficit may also contribute to the depression of myocardial contractility.

It is possible that the diabetic endothelial PARP pathway and the diabetic cardiomyopathy are interrelated: an impairment of the endothelial function may lead to global or regional myocardial ischemia, which may secondarily impair cardiac performance (43,44). It is noteworthy that the protective effect of PARP inhibition against diabetic cardiac dysfunction extended several weeks beyond the discontinuation of treatment; this observation may have important implications for the design of future clinical trials with PARP inhibitors. We have determined the
pharmacokinetic profile of intravenously injected PJ34 in rats, and we found that the compound has a plasma half-life of \( \frac{1}{2} \) h. No detectable PJ34 was found 24 h after the single intravenous injection of PJ34 in the animals (G.J. Southan, C.S., unpublished data). These observations support the view that the prolonged persistence of the effect of PJ34 is not related to the continued presence of the inhibitor, but, rather, may be related to the permanent interruption by the PARP inhibitor of positive feedback cycles of cardiac injury. Previous studies in various pathophysiological conditions have demonstrated that PARP inhibitors suppress positive feedback cycles of adhesion receptor expression and mononuclear cell infiltration, as well as intracellular oxidant generation (as discussed in 2,34,37,47). It is also conceivable that the degree of PARP activation may be more pronounced at the onset of the development of diabetic cardiovascular complications (as compared with a later stage of the disease) and when PARP is inhibited at an earlier time; this may result in more sustained beneficial effects. Although we cannot determine from the current studies whether the delayed

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**FIG. 6.** Evidence for PARP activation in diabetic mouse hearts. Immunohistochemical staining for poly(ADP-ribose) formation, an indicator of PARP activation, in control (A), diabetic (B), and PJ34-treated diabetic (C) mouse hearts. The evidence of enhanced poly(ADP-ribose) activity can be seen as dark, frequent, and widespread nuclear staining in panel B. Similar immunohistochemical profiles were seen in \( n = 4-5 \) hearts per group.

**FIG. 7.** Reversal of diabetes-induced endothelial dysfunction by pharmacological inhibition of PARP in diabetic NOD mouse vascular rings. Epinephrine-induced contractions (upper panel), acetylcholine-induced endothelium-dependent relaxation (middle panel), and sodium nitroprusside-induced endothelium-independent relaxations (lower panel). ■, control; ○, control + PJ34; □, diabetes; ●, diabetes + PJ34. Each point of the curve represents the means ± SE of 5–8 experiments in vascular rings. *\( P < 0.05 \) vs. C; #\( P < 0.05 \) vs. D.
In conclusion, our study provides experimental evidence that the PARP plays a central role in the pathogenesis of diabetic cardiovascular dysfunction. Further research is required to clarify whether PARP inhibition may exert beneficial effects against the development of various cardiovascular complications in diabetic patients.

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REFERENCES


