Poly(ADP-Ribose) Polymerase 1 Inhibition Improves Coronary Arteriole Function in Type 2 Diabetes Mellitus

Soo-Kyoung Choi, Maria Galán, Modar Kassan, Megan Partyka, Mohamed Trebak, Khalid Matrougui

Abstract—Type 2 diabetes mellitus (T2DM) is associated with microvascular dysfunction. We hypothesized that increased poly(ADP-ribose) polymerase 1 (PARP-1) activity contributes to microvascular dysfunction in T2DM. T2DM (db/db) and nondiabetic control (db/db+) mice were treated with 2 different PARP-1 inhibitors (INO-1001, 5 mg/kg per day and ABT-888, 15 mg/kg per day) for 2 weeks. Isolated coronary arterioles were mounted in an arteriograph. Pressure-induced myogenic tone was significantly potentiated, whereas endothelium-dependent relaxation was significantly attenuated in diabetic mice compared with control mice. These results were associated with decreased endothelial NO synthase phosphorylation and cGMP level and increased PARP-1 activity in coronary arterioles from diabetic mice compared with control mice. Interestingly, PARP-1 inhibitors significantly reduced the potentiation of myogenic tone, improved endothelium-dependent relaxation, restored endothelial NO synthase phosphorylation and cGMP, and attenuated cleaved PARP-1. These results were supported by in vitro studies indicating that downregulation of PARP-1 in mesenteric resistance arteries using PARP-1 short hairpin RNA lentiviral particles significantly improved endothelium-dependent relaxation in mesenteric resistance arteries from diabetic mice compared with control mice. The inhibition of NO synthesis by N\textsuperscripto\textendash nitro-L-arginine methyl ester (L-NAME) significantly reduced the endothelium-dependent relaxation in coronary arterioles and mesenteric resistance arteries from control and diabetic mice treated with PARP-1 inhibitors and PARP-1 short hairpin RNA lentiviral particles. In addition, we demonstrated that enhanced cleaved PARP-1, its binding to DNA, and DNA damage were reduced after PARP-1 inhibition in cultured endothelial cells stimulated with high glucose. We provide evidence that T2DM impairs microvascular function by an enhanced PARP-1 activity-dependent mechanism. Therefore, PARP-1 could be a potential target for overcoming diabetic microvascular complications. (Hypertension. 2012;59:00-00.)

Key Words: PARP-1 ■ endothelial dysfunction ■ coronary arteriole ■ myogenic tone ■ endothelium-dependent relaxation

Endothelium-dependent relaxation is a major function of the microcirculation. Physiological and pathological regulation of endothelium-dependent relaxation mechanism is fundamental and not yet determined. Clinically, most of the morbidity and mortality of diabetes mellitus are related to macrovascular and microvascular complications.\textsuperscript{1,2} Diabetes mellitus is a powerful risk factor for coronary artery disease, stroke, and peripheral arterial disease.\textsuperscript{3} It has been reported that endothelial function and myogenic tone are impaired in type 2 diabetes mellitus (T2DM).\textsuperscript{2,4} Limited studies to reveal the mechanisms underlying vascular dysfunction in diabetes mellitus have been conducted.

Poly(ADP-ribose) polymerase 1 (PARP-1) is an abundant nuclear enzyme involved in multiple DNA repair, such as single-strand breaks, double-strand breaks, and base excision.\textsuperscript{5,6} On binding to damaged DNA, PARP-1 cleaves nicotinamide adenine dinucleotide (NAD\textsuperscript{+}) into nicotinamide and ADP-ribose.\textsuperscript{7} When DNA damage is mild, PARP-1 participates in the DNA repair process. However, when DNA damage is extensive, PARP-1 is overactivated, which induces depletion of nicotine amide adenine dinucleotide and ATP levels and leads to cell dysfunction and death.\textsuperscript{8,9} PARP-1 activation can be triggered by oxidative stress and nitrosative stress, which affects multiple metabolic pathways, transcriptional regulation, and gene expression.\textsuperscript{10}

Although the involvement of PARP-1 is well documented in the field of cancer research\textsuperscript{11,12} and it has been reported that endothelial function was improved after PARP-1 inhibition in streptozotocin-induced type 1 diabetic mice,\textsuperscript{13} the role of PARP-1 and the downstream signaling in vascular function of T2DM mice are important questions and remain unanswered. Thus, the purpose of this study was to determine the potential therapeutic effect of PARP-1 inhibition to treat impaired vascular function in T2DM mice. To test our hypothesis, we treated mice with 2 novel potent PARP-1 inhibitors, INO-1001 and ABT-888.
Methods

Animal Models

Eight- to 10-week-old T2DM mice (db/db), which have a mutation in leptin receptor,13 and age-matched heterozygote control mice (db/db) were obtained from Jackson Laboratories. Mice were divided into 6 groups: (1) control mice with no treatment; (2) control mice who received PARP-1 inhibitor (INO-1001, 5 mg/kg per day from mini-osmotic pumps); (3) control mice who received another PARP-1 inhibitor (ABT-888, 15 mg/kg per day from mini-osmotic pumps); (4) diabetic mice without treatment; (5) diabetic mice treated with INO-1001 (5 mg/kg per day from mini-osmotic pumps); and (6) diabetic mice treated with ABT-888 (15 mg/kg per day from mini-osmotic pumps). The selection of the dose of PARP-1 inhibitors was based on previous studies.15,16 The chemical name of INO-1001 is 3-aminobenzamide, and it is an isoindolinone derivative and potent inhibitor of PARP-1 with chemosensitization and radiosensitization properties.17 ABT-888, velliparib, is (R)-2-(2-methylpyrrolidin-2-yl)-1H-benzo[d]imidazole-4-carboxamide and is also a potent PARP-1 inhibitor.18,19 These studies are conformed to the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Tulane University Institutional Animal Care.

Blood Glucose

Blood glucose measurements were obtained from tail blood samples using a blood glucose meter (Prestige Smart System HDE; Home Diagnostic) in all of the groups of mice after a 6-hours fast, as described previously.2

Blood Pressure

Arterial systolic blood pressure was measured in conscious mice using the CODA tail-cuff blood pressure system (Kent Scientific, Torrington, CT), as described previously.20

Insulin Resistance

Insulin resistance was determined by enzyme immunoassay using the Ultrasensitive Mouse Insulin ELISA protocol (Mercodia, Uppsala, Sweden), which estimates steady-state insulin resistance, as described previously.2

Preparation of Isolated Coronary Arterioles

After 2 weeks of treatment, mice were euthanized and coronary arterioles were isolated, cannulated with glass micropipettes, and perfused with physiological salt solution bubbled with a 95% O2 + 5% CO2 gas mixture. The vessels were pressurized to 50 mm Hg using pressure-servo control perfusion systems (Living Systems Instruments, St Albans, VT) for a 40-minute equilibration period. A video image analyzer, as described previously, monitored the vessel diameter.21 Intraluminal pressure was increased from 25 to 100 mm Hg in a stepwise manner to measure myogenic tone. At the end of the experiments, vessels were incubated with a calcium-free physiological salt solution to determine passive diameter. Myogenic tone is calculated as the percentage between active and passive diameters.12

Preparation of Isolated Mesenteric Arteries

Mesenteric resistance arteries were isolated and cleaned of fat and connective tissues. After incubation of arteries with PARP-1 short hairpin RNA (shRNA) lentiviral particle (Santa Cruz Biotechnology) for 4 hours, mesenteric resistance arteries were mounted in a myograph to determine endothelium-dependent relaxation, as described previously.22

Downregulation of PARP-1 Expression in Mesenteric Resistance Artery

Mesenteric resistance arteries were isolated and cleaned of fat and connective tissues. After incubation of arteries with PARP-1 short hairpin RNA (shRNA) lentiviral particle (Santa Cruz Biotechnology) for 4 hours, mesenteric resistance arteries were mounted in a myograph to determine endothelium-dependent relaxation, as described previously.22

Endothelial Cell Culture and Preparation of Nuclear Extracts

Mouse coronary arterioles endothelial cells (ECs) were purchased from CellProgen (San Pedro, CA) and cultured according to the manufacturer instructions using complete growth medium. Cultured ECs were starved for 24 hours in medium containing normal glucose and 1% of FBS and then stimulated with 25 mmol/L of d-glucose high glucose (HG) for 1 hour. PARP-1 inhibitors (INO-1001, 5 μmol/L and ABT-888, 5 μmol/L) were added 1 hour before the incubation with HG. Nuclear extracts were prepared as described previously.23

Immunocytochemistry

ECs were starved for 24 hours in normal glucose medium containing 1% of FBS and then exposed to PARP-1 inhibitors (INO-1001, 5 μmol/L and ABT-888, 5 μmol/L) 1 hour before the addition of HG for 1 hour. Cells were then washed in PBS, fixed in 4% paraformaldehyde, and incubated with anticleaved PARP-1 and total PARP-1 antibodies, followed by labeling with secondary antibody antimouse and antirabbit IgG conjugated to Alexa 594 and to Alexa 488 (Molecular Probes, Carlsbad, CA). After washing, the slides were mounted with Slow Fade Gold antifade reagent with 4’,6-diamidino-2-phenylindole (Molecular Probes), and immunofluorescent signal was visualized using a fluorescence microscope Eclipse 55i (×20; Nikon, Tokyo, Japan). The fluorescent signal was analyzed using the software NIS-Elements BR 3.0.

Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assays for PARP-1 were performed using nuclear extracts from cultured mouse coronary arteriole ECs. Nuclear cell extracts were incubated with double-strand unlabeled oligonucleotides (P350F: 5’-TCGAAAGTTGTTCTCTTTCGGAGAGGTTTCGGCC-3’; and inducible NO synthase: 5’-GAAGAAGATTATAATTATTAT- TTGCGT-3’), based on the oligonucleotide inducible NO synthase described by Yu et al.24 containing affinity PARP-1 binding sites. Briefly, both oligonucleotides were annealed to their antisense counterparts and added to 10 μg of ECs nuclear extracts, and the assay was performed using a fluorescence-based electrophoretic mobility shift assay kit (Molecular Probes). After 20 minutes of incubation at room temperature, the mixture was run on a 6% polyacrylamide nondenaturing gel (Novex TBE and DNA Retardation Gels; Invitrogen, Carlsbad, CA) in 0.5×TBE (4.45 mmol/L of Tris base, 4.45 mmol/L of boric acid, and 0.2 mmol/L of EDTA) for 90 minutes at room temperature. For supershift, nuclear extracts were preincubated for 5 minutes with anti–PARP-1 (Santa Cruz MA), and total and cleaved PARP-1 using specific antibodies (1:1000 dilution; Cell Signaling). Blots were stripped and then reprobed with the GAPDH antibody (1:2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) to verify the equal loading between the samples.
Biotechnology), and 10 μg of nuclear extracts from cells overexpressing PARP-1 were used as a binding positive control.

**DNA Damage Analysis by Comet Assay**

The presence of DNA damage was examined by single-cell electrophoresis (Comet assay) using the manufacturer’s protocol (OxiSelect Comet Assay kit; Cell Biolabs, Inc, San Diego, CA). Briefly, ECs were stimulated with normal glucose or HG for 24 hours with or without PARP-1 inhibitors. Cells were then harvested and washed with PBS. Cell suspension was mixed with agarose at 37°C, and then 75 μL of this mixture was instantly added to comet slides. Slides were immersed in lysis buffer at 4°C for 1 hour and then replaced with prechilled alkaline buffer and placed at 4°C for 30 minutes followed by electrophoresis (35 V for 15 minutes) in a horizontal electrophoresis chamber filled with Tris borate EDTA buffer. Slides were transferred to a container filled with cold water for 2 to 4 minutes and then placed in 70% ethanol for 5 minutes and air dried overnight at room temperature. After staining with Vista Green DNA dye for 15 minutes at room temperature, comets were observed by fluorescent microscopy (Nikon) with ×20 magnification. The images were analyzed using the software NIS-Elements BR 3.0 (Nikon).

**Statistical Analysis**

Results are expressed as mean±SEM. One-way or 2-way ANOVA was used to compare each parameter when appropriate. Comparisons between groups were performed with t tests when the ANOVA test was statistically significant. Values of P<0.05 were considered significant. Differences between specified groups were analyzed using the Student t test (2-tailed) for comparing 2 groups, with P<0.05 considered statistically significant.

**Results**

**Effect of PARP-1 Inhibition on Blood Glucose, Insulin Levels, Body Weight, and Systolic Blood Pressure**

Body weight, blood glucose, and insulin levels were increased in diabetic mice compared with control mice and were not affected by INO-1001 or ABT-888 treatments (Figure 1A through 1C). Body weight, blood glucose, and insulin levels were increased in diabetic mice compared with control mice and were not affected by INO-1001 or ABT-888 treatments (Figure 1A through 1C). Blood pressure was similar in all of the groups of mice, which indicates that T2DM (db/db/db) mice are normotensive (Figure 1D).

**Effect of PARP-1 Inhibition on Myogenic Tone and Endothelium-Dependent Relaxation in Coronary Arterioles**

Myogenic tone was significantly increased in coronary arterioles from T2DM mice compared with control mice and were not affected by INO-1001 or ABT-888 treatments (Figure 2A) and was significantly reduced after PARP-1 inhibition (Figure 2A). The passive diameters at 50 mm Hg in all groups of mice were not statistically different (115.5±3.4 μm for nontreated control mice; 120.2±10.1 μm for control mice treated with INO-1001; 117.2±10.1 μm for control mice treated with ABT-888; 114.2±12.1 μm for nontreated dia-
Figure 2. Effect of poly(ADP-ribose) polymerase 1 (PARP-1) inhibitions on myogenic tone and endothelium-dependent relaxation in coronary arterioles. A, Pressure-induced myogenic responses in coronary arteries from all of the groups treated with or without PARP-1 inhibitors (INO-1001, 5 mg/kg per day, or ABT-888, 15 mg/kg per day). *P<0.05 for diabetic vs control mice; ‡P<0.05 for diabetic vs diabetic mice treated with INO-1001; †P<0.05 for diabetic vs diabetic mice treated with ABT-888 (n=5). B, Changes in diameter in response to dose response (10^{-9} to 10^{-5} mol/L) of acetylcholine in coronary artery from all of the groups of mice. *P<0.05 for diabetic vs control mice; ‡P<0.05 for diabetic vs diabetic mice treated with INO-1001; †P<0.05 for diabetic vs diabetic mice treated with ABT-888 (n=5). C, Changes in diameter in response to dose response (10^{-9} to 10^{-5} mol/L) of acetylcholine with pretreatment of exogenous NO synthase inhibitor (L-NAME) in coronary arterioles from all of the groups of mice. *P<0.05, n=5. D, Western blot analysis for phosphorylated and total eNOS from all of the groups. *P<0.05 for diabetic vs control mice; ‡P<0.05 for diabetic vs diabetic mice treated with INO-1001; †P<0.05 for diabetic vs diabetic mice treated with ABT-888 (n=5). E, cGMP levels from
Figure 2 (Continued). all mice in the group. *P<0.05 for control vs diabetic mice; †P<0.05 for control mice vs control mice treated with ABT-888; §P<0.05 for diabetic vs diabetic mice treated with ABT-888; ‡P<0.05 for diabetic vs diabetic mice treated with INO-1001 (n=5). F. Detection of cleaved PARP-1 and total PARP-1 proteins from all of the groups. Bars indicate the quantification of the results of eNOS Western blot data were supported by cGMP measurements, indicating that PARP-1 inhibition restores cGMP level to the normal level (Figure 2E).

Effect of PARP-1 Inhibition on Cleaved and Total PARP-1 Levels on Endothelium-Dependent Relaxation in Coronary Arteriole

Cleaved PARP-1 level was increased in heart tissues from diabetic mice compared with control mice. The treatment of L-NAME were similar in all of the groups of mice (Figure 2C). In Figure 2D, phosphorylated eNOS at Ser635 and total eNOS were standardized to GAPDH. The results were also presented as follows: total eNOS was standardized to GAPDH and then phosphorylated eNOS was standardized to total eNOS/GAPDH (phosphorylated eNOS/total eNOS/GAPDH). The results of eNOS Western blot data were supported by cGMP measurements, indicating that PARP-1 inhibition restores cGMP level to the normal level (Figure 2E).

Figure 3. Effects of poly(ADP-ribose) polymerase 1 (PARP-1) short hairpin RNA (shRNA) lentivirus particle on endothelium-dependent relaxation in mesenteric resistance arteries (MRAs). A, Changes in diameter in response to dose response (10^{-8} to 10^{-4} mol/L) of acetylcholine in MRAs from control mice; *P<0.05 (n=5). B, Changes in diameter in response to dose response (10^{-8} to 10^{-4} mol/L) of acetylcholine in MRAs from diabetic mice; *P<0.05 (n=5). C, Western blot analysis showing the effects of PARP-1 shRNA lentivirus particle on the expression of PARP-1 protein; *P<0.05 (n=5).

Betic mice; 118±9.0 µm for diabetic mice treated with INO-1001; and 114.2±7.9 µm for diabetic mice treated with ABT-888). Thromboxane analog, U46619, induced similar contractions in coronary arterioles from all of the groups (data not shown). Endothelium-dependent relaxation was significantly reduced in coronary arterioles from diabetic mice compared with control mice. Interestingly, endothelium-dependent relaxation was significantly improved in coronary arterioles from diabetic mice treated with PARP-1 inhibitors. The endothelium-dependent relaxation of coronary arterioles was shifted to the left in control mice treated with ABT-888 (Figure 2B). The -log EC_{50}S of acetylcholine in all of the groups of mice were not statistically different (5.96±0.18 for nontreated control mouse; 6.43±0.13 for control mice treated with INO-1001; 7.44±0.11 for control mice treated with ABT-888; 6.9±0.08 for nontreated diabetic mice; 6.45±0.2 for diabetic mice treated with INO-1001; and 6.18±0.31 for diabetic mice treated with ABT-888). The endothelium-dependent relaxation in coronary arterioles in the presence of NOS synthase inhibitor (L-NAME) was similar in all of the groups of mice (Figure 2C). The -log EC_{50}S for acetylcholine in the presence of L-NAME were similar in all of the groups of mice (7.00±0.49 for nontreated control mouse; 7.66±0.29 for control mice treated with INO-1001; 7.00±0.34 for control mice treated with ABT-888; 7.50±0.13 for nontreated diabetic mice; 6.06±0.16 for diabetic mice treated with INO-1001; and 7.04±0.27 for diabetic mice treated with ABT-888). The endothelium-dependent relaxation in the presence of L-NAME was associated with reduced eNOS phosphorylation at Ser635 and expression in coronary arterioles in diabetic mice (Figure 2D). Importantly, eNOS phosphorylation and expression were restored in coronary arterioles after treatment with PARP-1 inhibitors (Figure 2D). In Figure 2D, phosphorylated eNOS at Ser635 and total eNOS were standardized to GAPDH. The results were also presented as follows: total eNOS was standardized to GAPDH and then phosphorylated eNOS was standardized to total eNOS/GAPDH (phosphorylated eNOS/total eNOS/GAPDH). The results of eNOS Western blot data were supported by cGMP measurements, indicating that PARP-1 inhibition restores cGMP level to the normal level (Figure 2E).
Figure 4 (Continued).

A

B

C

D

Supershift
Bandshift
PARP-1-P30ds

Figure 4 (Continued).
with INO-1001 or ABT-888 did not affect cleaved PARP-1 levels in the control group. However, INO-1001 and ABT-888 significantly decreased cleaved PARP-1 levels in diabetic mice (Figure 2F). Total PARP-1 level was also increased in diabetic mice and was not affected by PARP-1 inhibitors (Figure 2F).

Effect of PARP-1 Downregulation by PARP-1 shRNA Lentiviral Particle on Endothelium-Dependent Relaxation in Mesenteric Resistance Artery
We downregulated PARP-1 expression in mesenteric resistance arteries by incubating them with PARP-1 shRNA lentiviral particles. PARP-1 shRNA lentiviral particles significantly reduced PARP-1 expression, as evidenced by Western blot analysis (Figure 3A). Interestingly, downregulation of PARP-1 expression significantly improved endothelium-dependent relaxation in mesenteric resistance arteries in diabetic mice, whereas no effect was observed in mesenteric resistance arteries from control mice (Figure 3B and 3C). The inhibition of NO synthesis greatly reduced endothelium-dependent relaxation in mesenteric resistance arteries from diabetic mice and control mice subjected to PARP-1 shRNA lentiviral particles (Figure 3B and 3C).

Effect of HG on PARP-1 Activity, Its Binding to DNA, and DNA Damage
Primary cultured ECs from coronary arterioles with HG stimulation showed an increase in cleaved PARP-1 evidenced by Western blot analysis in nuclear fractions, immunostaining, and electrophoretic mobility shift Assay analysis (Figure 4). The level of cleaved PARP-1 was increased in the nuclear extracts after incubation of ECs with HG and was reduced in the presence of INO-1001 and ABT-888 (Figure 4A). In the cytoplasmic fraction, cleaved PARP-1 was not detected (data not shown). Total PARP-1 level was also increased in nuclear extracts after incubation with HG but was not affected by PARP-1 inhibitors (Figure 4A). We also demonstrated using an immunofluorescence signal that cleaved PARP-1 was increased after incubation with HG and was reduced when cells were pretreated with PARP-1 inhibitors (Figure 4B). Similarly, total PARP-1 signal was also increased in the presence of HG and was not altered after pretreatment with PARP-1 inhibitors (Figure 4B). Nuclear extracts of ECs incubated with HG showed higher DNA-binding activity. Pretreated cells with PARP-1 inhibitors induced a marked decrease in the DNA-binding activity of PARP-1 (Figure 4C). The effect of HG and PARP-1 inhibition on DNA strand breaks was determined using the Comet single cell electrophoresis assay. After stimulation of ECs with HG for 24 hours, there was a significant increase in the length of the DNA tails by 2-fold compared with control cells (Figure 4D). The pretreatment of ECs with PARP-1 inhibitors significantly reduced the length of tails induced by HG (Figure 4D).

Discussion
Impaired microvascular function is an early event in the development of cardiovascular diseases. In this study, we found that elevated PARP-1 activity is responsible for microvascular dysfunction in T2DM mice. Importantly, the inhibition of PARP-1 activity reduces myogenic tone and improves endothelium-dependent relaxation in coronary arterioles and mesenteric resistance arteries in T2DM mice. It has been reported that PARP-1 isoform is implicated in the pathogenesis of diabetes mellitus and diabetic complications including peripheral neuropathy. However, the role and mechanisms of PARP-1 upregulation in microvascular dysfunction in T2DM remain unclear.

In this study, we found that myogenic tone was elevated and endothelium-dependent relaxation was attenuated in diabetic mice. This finding was associated with a decrease in eNOS phosphorylation, expression, and cGMP level. Our data are in agreement with previous data showing elevated myogenic tone and impaired endothelial function in diabetic mice. Interestingly, PARP-1 inhibition restored eNOS phosphorylation at Ser635 and expression and cGMP level, associated with normalized myogenic tone and improved endothelium-dependent relaxation in diabetic mice. These data suggest that exacerbated PARP-1 activation is responsible for microvascular dysfunction. It has been reported that PARP-1–mediated endothelial dysfunction in diabetes mellitus is related to hyperglycemia.13 HG induces a significant PARP-1–dependent change in ATP content and NADPH. Previous study suggested that the depletion of nicotine amide adenine dinucleotide in ECs exposed to HG is directly responsible for the inhibition of eNOS activity and the impairment of vascular endothelium-dependent relaxation in diabetes mellitus. Our data are in agreement because eNOS activity was significantly reduced and treatment with PARP-1 inhibitors recovered eNOS activity in T2DM mice. These data suggest that eNOS expression and phosphorylation at Ser635 are regulated by PARP-1 activity; however, the mechanism is still unclear.

It is known that, during extensive cell damage, PARP-1 can be cleaved into 2 fragments, which are p89 and p24. In the present study, we found an increase in the 24-kDa fragment of cleaved PARP-1 from diabetic mice compared
with control mice, which are suppressed by PARP-1 inhibitors, whereas total PARP-1 levels were not affected.

To further demonstrate the role of PARP-1 in microvascular function, we downregulated PARP-1 expression and found an improvement in endothelium-dependent relaxation in mesenteric resistance arteries from diabetic mice. Thus, our data suggest that, although DNA repair is withheld, the overall tissue damage exposed to hyperglycemia may be reduced by PARP-1 inhibition. These findings are in agreement with previous studies reporting that PARP-1–deficient mice are resistant to streptozotocin-induced diabetes mellitus.34,35

To support the functional studies, we performed in vitro studies with ECs exposed to HG to simulate in vivo condition of diabetes mellitus. Nuclear fractions of ECs were subjected to Western blot analysis, and the results indicated an increase in cleaved PARP-1 in cells exposed to HG. Cleaved PARP-1, detected in nuclear fraction but not in the cytoplasmic fraction, was increased in ECs exposed to HG and was decreased in the presence of INO-1001 and ABT-888. The expression of total PARP-1 was also augmented in nuclear extracts but was not altered with PARP-1 inhibitors. These data correlate with electrophoretic mobility shift assay analysis, indicating that HG stimulation showed higher DNA-binding activity. Pretreated cells with INO-1001 and ABT-888 reduced the DNA-binding activity of PARP-1. According to a previous report, a significant poly(ADP-ribose) immunostaining was found in microvessels of diabetic retinae.36 In the present study, we demonstrated that cleaved PARP-1 and total PARP-1 were increased in cells stimulated with HG. Inhibition of PARP-1 activity significantly reduced cleaved PARP-1 fluorescence in the nuclei of cells stimulated with HG, but it did not affect the total PARP-1. These data correlate with our in vivo study and are also in agreement with previous studies reporting that pretreatment of animals with PARP-1 inhibitors reduced PARP-1 activity and protected against streptozotocin-induced β-cell necrosis and hyperglycemia.37,38

The comet assay has been extensively used to detect DNA damage induced by various stimuli.39 In our study, pretreatment with PARP-1 inhibitors reduced the length of the comet tail induced by HG, suggesting that the reduction in the exacerbation of PARP-1 activity could protect the cells from excessive DNA damage.

Our findings indicate that elevated PARP-1 activity is responsible for impaired endothelium-dependent relaxation and potentiation of myogenic tone in T2DM. Importantly, in vivo and in vitro inhibition of PARP-1 improved microvascular function in T2DM. Therefore, PARP-1 inhibition could be a potential strategy to overcome T2DM-induced vascular dysfunction.

**Perspectives**

Diabetes mellitus is a metabolic disease associated with vascular complication, including impaired microvascular endothelium-dependent relaxation and myogenic reactivity. Most clinical studies indicate that patients with T2DM are at high risk for cardiovascular diseases. Although the clinical management of T2DM has advanced substantially, cardiovascular diseases in patients with T2DM still constitute a major and increasing health burden worldwide. Despite the fact that treatments have progressed, the development of novel effective treatments for patients with vascular complications in T2DM remains a major research goal. Therefore, there is a significant medical need to develop novel therapies to restore microvascular endothelial function, especially in patients with T2DM. Our results indicate that elevated PARP-1 activity impairs vascular function in T2DM mice. Interestingly, inhibition of PARP-1 activity improves microvascular function through restoration of the eNOS-cGMP pathway. Therefore, PARP-1 could be a potential target for a therapeutic strategy to improve vascular function in diabetes mellitus.

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**Disclosures**

None.

**References**


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