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Poly(ADP-ribose) polymerase (PARP) inhibition counteracts multiple manifestations of kidney disease in long-term streptozotocin-diabetic rat model

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ABSTRACT

Evidence for the important role for poly(ADP-ribose) polymerase (PARP) in the pathogenesis of diabetic nephropathy is emerging. We previously reported that PARP inhibitors counteract early Type 1 diabetic nephropathy. This study evaluated the role for PARP in kidney disease in long-term Type 1 diabetes. Control and streptozotocin-diabetic rats were maintained with or without treatment with the PARP inhibitor 10-(4-methyl-piperazin-1-ylmethyl)-2H-7-oxa-1,2-diaza-benzo[de] anthracen-3-one (GPI-15,427, Eisai Inc.), 30 mg kg^{-1} d^{-1}, for 26 weeks after first 2 weeks without treatment. PARP activity in the renal cortex was assessed by Western blot analysis of poly(ADP-ribosyl)ated proteins. Urinary albumin, isoprostane, and 8-hydroxy-2′-deoxyguanosine excretion, and renal concentrations of transforming growth factor-β_1, vascular endothelial growth factor, soluble intercellular adhesion molecule-1, fibronectin, and nitrotyrosine were evaluated by ELISA, and urinary creatinine and renal lipid peroxidation products by colorimetric assays. PARP inhibition counteracted diabetes-associated increase in renal cortex poly(ADP-ribosyl)ated protein level. Urinary albumin, isoprostane, and 8-hydroxy-2′-deoxyguanosine excretions and urinary albumin/creatinine ratio were increased in diabetic rats, and all these changes were at least partially prevented by GPI-15,427 treatment. PARP inhibition counteracted diabetes-induced renal transforming growth factor-β_1, vascular endothelial growth factor, and fibronectin, but not soluble intercellular adhesion molecule-1 and nitrotyrosine, accumulations. Lipid peroxidation product concentrations were indistinguishable among control and diabetic rats maintained with or without GPI-15,427 treatment. In conclusion, PARP activation plays an important role in kidney disease in long-term diabetes. These findings provide rationale for development and further studies of PARP inhibitors and PARP inhibitor-containing combination therapies, for prevention and treatment of diabetic nephropathy.

1. Introduction

Diabetic nephropathy develops in 30–40% of patients with both Type 1 and Type 2 diabetes mellitus within 20–25 years after the onset of diabetes [1,2]. Diabetes now accounts for at least ~35% of all new cases of end-stage renal disease in the United States [3], and diabetic patients make up the fastest growing group of renal dialysis and transplant recipients. Diabetic nephropathy is also associated with elevated blood pressure, increased incidence of coronary heart disease, stroke, peripheral arterial occlusive disease, other microvascular complications of diabetes like diabetic retinopathy or diabetic foot, and premature mortality [4]. A recent study revealed that subjects with both Type 1 and Type 2 diabetes and initial stage of nephropathy (manifested by microalbuminuria) already have increased cardiovascular risk, morbidity and mortality [1,5].

The pathogenesis of diabetic nephropathy has extensively been studied in animal models of diabetes, and involves complex interactions between haemodynamic and metabolic factors [6]. The haemodynamic factors include increased systemic and intraglomerular pressure and activation of various vasoactive hormone pathways, e.g., the renin–angiotensin and endothelin systems [6]. The metabolic mechanisms include increased sorbitol pathway activity [7,8], non-enzymatic glycation/glycoxidation [8–10], activation of protein kinase C, 12/15-lipoxygenase, and hexosamine pathway [11–14]. Evidence for the important role for oxidative stress in diabetic kidney disease is emerging [15–19]. Oxidative stress is linked to activation of mitogen-activated

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protein kinases (MAPKs) [20], the nuclear transcription factor NF-κB [21], and upregulation of growth factors such as cytokines [22], and vascular endothelial growth factor (VEGF) [23] implicated in diabetic renal disease [24].

One of the most important phenomena closely linked to oxidative stress is activation of poly(ADP-ribose) polymerase (PARP), known to lead to NAD^+ depletion and energy failure [25], activation of non-enzymatic glycation and protein kinase C [26], impairment in signal transduction mechanisms [27], and changes in transcriptional regulation and gene expression [28]. Evidence for the important role for PARP activation in the pathogenesis of diabetic complications including endothelial and myocardial dysfunction, peripheral and autonomic neuropathy, retinopathy, and cataract is emerging [29–33]. PARP activation has been implicated in diabetic nephropathy in leptin receptor-deficient (*db*/*db*) mice, a model of Type 2 (non-insulin-dependent) diabetes [34]. Furthermore, we recently reported that PARP activation in the renal cortex contributes to enhanced production of transforming growth factor-β, endothelin-1, and vascular endothelial growth factor, as well as enhanced oxidative-nitrosative stress, advanced glycation end-product formation, and pro-inflammatory response, and plays an important role in albuminuria, podocyte loss, and mesangial expansion associated with early nephropathy in the streptozotocin-diabetic rat model [35]. The purpose of the present study was to evaluate the effect of PARP inhibition on indices of kidney disease associated with chronic Type 1 diabetes.

2. Materials and methods

2.1. Reagents

Unless otherwise stated, all chemicals were of reagent-grade quality, and were purchased from Sigma Chemical Co., St. Louis, MO. GPI-15,427 was obtained from Eisai Inc., Baltimore, MD. Mouse monoclonal anti-poly(ADP-ribose) antibody was purchased from Trevigen, Inc., Gaithersburg, MD.

2.2. Animals

The experiments were performed in accordance with regulations specified by the National Institutes of Health "Principles of Laboratory Animal Care, 1985 Revised Version" and Pennington Biomedical Research Center Protocol for Animal Studies. Male Wistar rats (Charles River, Wilmington, MA), body weight 250–300 g, were fed a standard rat chow (PMI Nutrition Int., Brentwood, MO) and had access to water *ad libitum*. STZ-diabetes was induced as described [35]. Blood samples for glucose measurements were taken from the tail vein ~48 h after the STZ injection and the day prior to the study termination. All rats with blood glucose levels ≥13.8 mM were considered diabetic. Diabetic rats were maintained on suboptimal doses of insulin (~1–2 U/every second day) to prevent ketoacidosis and weight loss. The experimental groups comprised control and diabetic rats treated with or without the PARP inhibitor GPI-15,427 (formulated as mesilate salt, 30 mg kg^{-1} d^{-1}, in the drinking water), for 26 weeks after first 2 weeks without treatment. An initial 2-week period without treatment was introduced to avoid β-cell regeneration and alleviation of hyperglycemia which is known to occur when PARP inhibitors are administered together with streptozotocin or shortly after induction of diabetes [25]. At the end of the study, rats were placed in individual metabolic cages (Lab Products Inc., Seaford, DE) and urine collected for 24 h. Urine specimen were centrifuged at 12,000 × *g* (4 °C, 10 min) and frozen for subsequent assessment of albumin, isoprostane, and 8-hydroxy-2′-deoxyguanosine by ELISA and creatinine by spectrophotometric procedure based on the Jaffe reaction.

2.3. Anesthesia, euthanasia and tissue sampling

The animals were sedated by CO_2, and immediately sacrificed by cervical dislocation. Glycosylated hemoglobin was measured using A1cNow INVIEW Multi-test A1C system (Metrika, Sunnyvale, CA). Kidneys were immediately frozen in liquid nitrogen for subsequent Western blot analysis of poly(ADP-ribosyl)ated proteins, ELISA measurements of renal transforming growth factor β_1 (TGF-β_1), vascular endothelial growth factor (VEGF), fibronectin, nitrotyrosine (NT), and soluble intercellular adhesion molecule-1 (iCAM-1) concentrations, and colorimetric assessment of renal malondialdehyde (MDA) and MDA plus 4-hydroxyalkenal (4-HA) concentrations.

2.4. Specific methods

2.4.1. Urinary albumin, creatinine, isoprostane and 8-hydroxy-2′-deoxyguanosine excretion

Urinary albumin, isoprostane, and 8-hydroxy-2′-deoxyguanosine excretions were assessed by ELISA. The Nephrat kit (Exocell, Philadelphia, PA), Urinary Isoprostane ELISA kit (Oxford Biomedical Research, Oxford, MI) and 8-hydroxy-2′-deoxy Guanosine EIA Kit (Cayman Chemical Company, Ann Arbor, MI) were used for measurements of albumin, isoprostane, and 8-hydroxy-2′-deoxyguanosine, respectively. Urinary creatinine was measured spectrophotometrically, using Creatinine Parameter assay kit (R&D Systems, Minneapolis, MN). The assays were performed in accordance with the manufacturers' instructions.

2.4.2. TGF-β_1, VEGF, fibronectin, MDA, MDA plus 4-HA, NT, and iCAM-1 concentrations

For measurements of TGF-β_1, iCAM-1 and NT concentrations, renal cortex samples were homogenized on ice in RIPA buffer (1:10, w/v) containing 50 mM Tris–HCl, pH 7.2; 150 mM NaCl; 0.1% sodium dodecyl sulfate; 1% NP-40; 5 mM EDTA; 1 mM EGTA; 1% sodium deoxycholate and the protease/phosphatase inhibitors leupeptin (10 μg/ml), aprotinin (20 μg/ml), benzamidine (10 mM), phenylmethylsulfonyl fluoride (1 mM), sodium orthovanadate (1 mM). Homogenates were sonicated (3× 5 s) and centrifuged at 14,000 × *g* (4 °C, 20 min). TGF-β_1 and NT concentrations were measured with the Quantikine mouse/rat/porcine/canine TGF-β_1 kit (R&D Systems, Minneapolis, MN) and the OxiSelect Nitrotyrosine ELISA kit (Cell Biolabs, San Diego, CA).

For VEGF measurements, renal cortex samples were homogenized in 20 mM phosphate-buffered saline (PBS), pH 7.4 (1:5, w/v), on ice. Homogenate was used for VEGF measurements with the Quantikine Rat VEGF ELISA kit (R&D Systems, Minneapolis, MN).

For fibronectin, MDA, and MDA + HA measurements, renal cortex samples were homogenized in 20 mM PBS, pH 7.4 (1:10, w/v), on ice. Homogenate was centrifuged at 14,000 × *g* (4 °C, 20 min). Supernatant fibronectin concentrations were measured with the AssayMax Rat Fibronectin ELISA kit (AssayPro, St. Charles, MO). Supernatant MDA and MDA + 4-HA concentrations were measured with the Bioxytech LPO-586 kit (Oxis International, Inc., Foster City, CA). All measurements were performed according to the manufacturers' instruction.

For iCAM-1 measurements, kidney tissue was homogenized (1:10, w/v) in 20 mM PBS, pH 7.4, containing the protease inhibitors (10 μg/ml leupeptin, 20 μg/ml aprotinin, 10 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 1 μg/ml pepstatin), on ice. Homogenates were centrifuged at 12,000 × *g* (4 °C, 20 min). Supernatant soluble iCAM concentrations were measured with the Quantikine rat soluble iCAM kit (R&D Systems, Minneapolis, MN). Protein was measured with the bicinchoninic acid protein assay (Pierce Biotechnology, Rockford, IL).

Table 1
Initial and final body weights, blood glucose and glycosylated hemoglobin concentrations in control and diabetic rats maintained with and without PARP inhibitor treatment.

Variable	Control	Control + GPI-15,427	Diabetic	Diabetic + GPI-15,427
Initial body weight (g)	293 ± 3.4	296 ± 5.3	263 ± 4.2	264 ± 3.9
Final body weight (g)	591 ± 23	545 ± 8	377 ± 19**	362 ± 16**
Initial blood glucose (mmol/l)	5.58 ± 0.08	5.32 ± 0.1	22.7 ± 1.4**	23.4 ± 0.4**
Final blood glucose (mmol/l)	5.99 ± 0.22	6.22 ± 0.11	18.4 ± 1**	18.9 ± 2.6**
HbA_1c (%)	5.34 ± 0.07	5.12 ± 0.06	11.8 ± 0.3**	11.4 ± 0.5**

Data are Means ± SEM, $n = 8–10$ per group.
** $p < 0.01$ vs controls.

2.4.3. Western blot analysis

Western blot analysis of poly(ADP-ribosyl)ated proteins was performed as described previously [36]. Protein bands were visualized with the Amersham ECL Western blotting detection reagents and analysis system (GE Healthcare, Buckinghampshire, UK). Membranes were then stripped and reprobed with β-actin antibody to verify equal protein loading. The data were quantified by densitometry (Quantity One 4.5.0 software, Bio-Rad Laboratories, Richmond, CA).

2.5. Statistical analysis

The results are expressed as Mean ± SEM. Data were subjected to equality of variance *F*-test, and then to log transformation, if necessary, before one-way analysis of variance. Where overall significance ($p < 0.05$) was attained, individual between group comparisons were made using the Student–Newman–Keuls multiple range test. Significance was defined at $p \leq 0.05$. When between-group variance differences could not be normalized by log transformation (datasets for body weights and plasma glucose), the data were analyzed by the nonparametric Kruskal–Wallis one-way analysis of variance, followed by the Bonferroni/Dunn or Fisher's PLSD tests for multiple comparisons.

3. Results

3.1. PARP inhibition did not affect weight gain or glycemia in either non-diabetic or diabetic rats

The initial (prior to STZ administration) body weights were similar in control and diabetic rats maintained with or without GPI-15,427 treatment. The final body weights were similarly reduced in untreated and PARP inhibitor-treated diabetic rats compared with the control group (Table 1). Initial blood glucose concentrations were increased 4.1- and 4.2-fold in untreated and GPI-15,427-treated diabetic rats, respectively, compared with non-diabetic controls. Final blood glucose concentrations were 3.1- and 3.2-fold higher in untreated and GPI-15,427-treated diabetic rats than in non-diabetic controls. Final HbA_1c concentrations were increased 2.2- and 2.1-fold in untreated and GPI-15,427-treated diabetic rats compared with non-diabetic controls. PARP inhibition did not affect either weight gain, or level of glycemia in non-diabetic rats.

3.2. GPI-15,427 partially suppressed diabetes-induced poly(ADP-ribosyl)ated protein accumulation in the renal cortex

Poly(ADP-ribosyl)ated protein expression was increased 1.5-fold in untreated diabetic rats compared with non-diabetic controls, and this increase was partially prevented by GPI-15,427 treatment (to 123.8% of control value, Fig. 1A and B). PARP inhibition did not affect poly(ADP-ribosyl)ated protein expression in non-diabetic control rats.

3.3. PARP inhibition counteracted diabetes-induced albuminuria

Urinary albumin excretion was increased 7.8-fold in untreated diabetic rats compared with non-diabetic controls, and this increase was partially prevented by GPI-15,427 (Fig. 2A). In a similar fashion, urinary albumin/creatinine ratio was increased in untreated diabetic group (Fig. 2B). This ratio was reduced by a

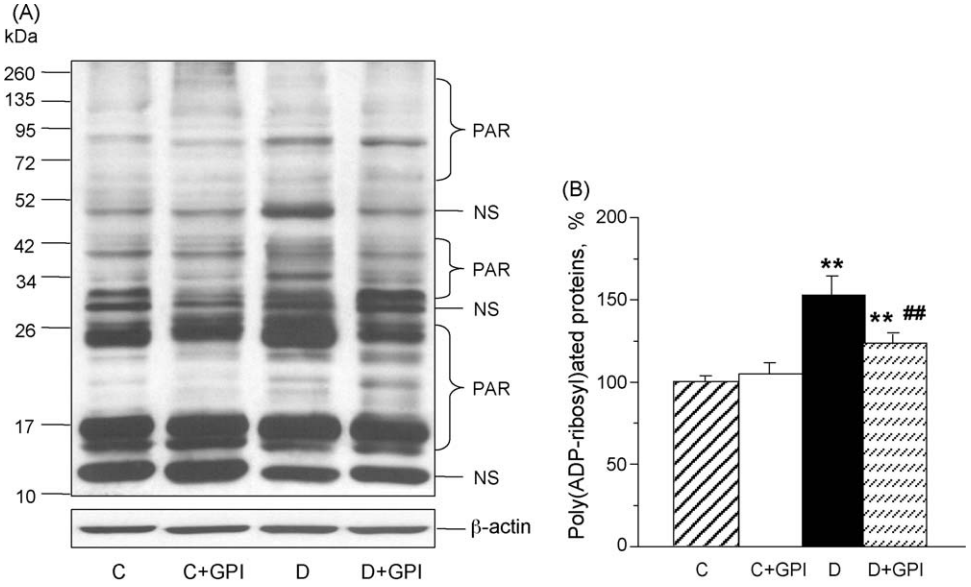

Fig. 1. The PARP inhibitor GPI-15,427, at 30 mg kg^{-1} d^{-1}, partially suppresses diabetes-induced accumulation of poly(ADP-ribosyl)ated proteins in the renal cortex as manifested by representative Western blot analysis of renal poly(ADP-ribosyl)ated proteins (A) and poly(ADP-ribosyl)ated protein content (densitometry, B). C – control group; D – diabetic group. PAR – poly(ADP-ribosyl)ated proteins. NS – non-specific band. Mean ± SEM, $n = 5–7$ per group. **$p < 0.01$ vs control group; ##$p < 0.01$ vs untreated diabetic group.

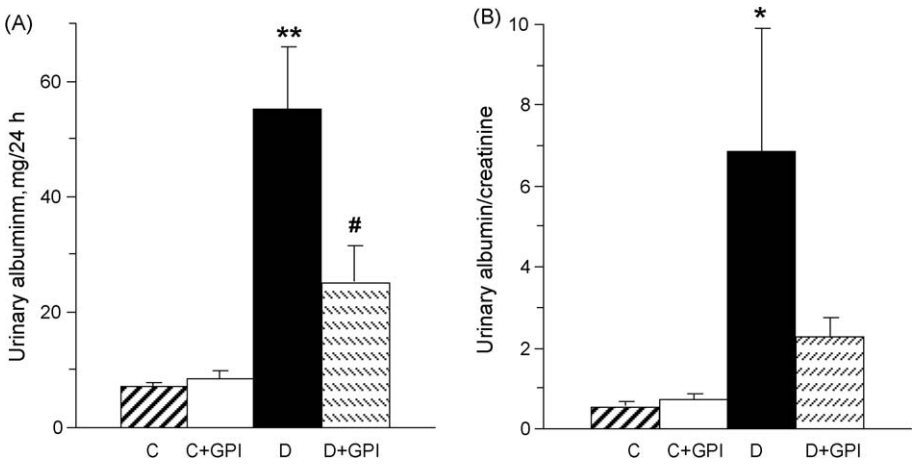

Fig. 2. PARP inhibition reduces, but does not completely prevent, diabetes-associated albuminuria (A) and increase in urinary albumin/creatinine ratio. C – control group; D – diabetic group. Mean ± SEM, n = 8–10 per group. $^{*}p < 0.05$, $^{**}p < 0.01$ vs controls; $^{\#}p < 0.05$ vs untreated diabetic group.

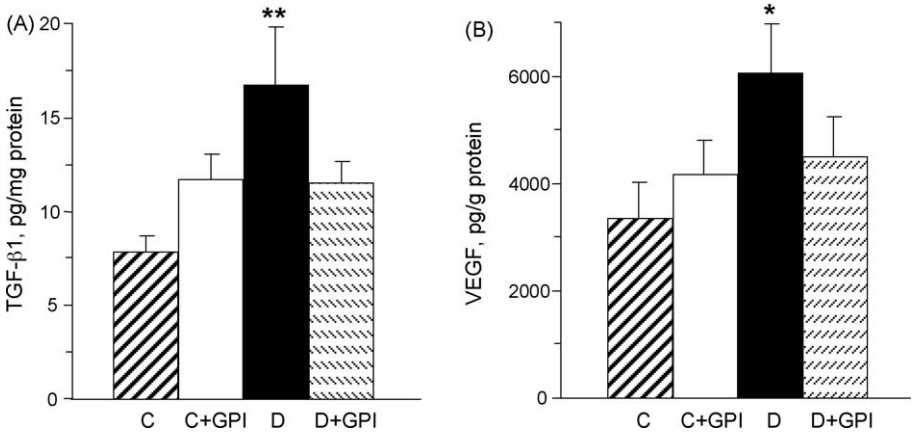

Fig. 3. PARP inhibition counteracts diabetes-induced accumulation of TGF-β_1 (A) and VEGF (B) in the renal cortex. C – control group; D – diabetic group. Mean ± SEM, n = 7–10 per group. $^{*}p < 0.05$, $^{**}p < 0.01$ vs controls.

PARP inhibitor treatment to the level that was not significantly different from those in either control or untreated diabetic rats (Fig. 2B).

3.4. PARP inhibition counteracted diabetes-induced growth factor imbalances and fibronectin accumulation in the renal cortex

Diabetic rats displayed 2.1- and 1.8-fold increases in renal TGF-β_1 and VEGF concentrations, respectively, compared with non-diabetic controls (Fig. 3A and B). A PARP inhibitor treatment reduced the concentrations of both growth factors to the levels that were not significantly different from those in either control or untreated diabetic groups. GPI-15,427 did not significantly affect renal TGF-β_1 and VEGF concentrations in non-diabetic rats. Renal fibronectin concentration was increased 1.6-fold in untreated diabetic rats compared with controls (Fig. 4), and this increase was essentially prevented by a PARP inhibitor treatment.

3.5. PARP inhibition counteracted diabetes-induced systemic, but not renal, oxidative stress and did not affect renal iCAM accumulation

Urinary isoprostane (Fig. 5A) and 8-hydroxy-2′-deoxyguanosine (Fig. 5B) excretions were increased 17.5- and 1.5-fold in

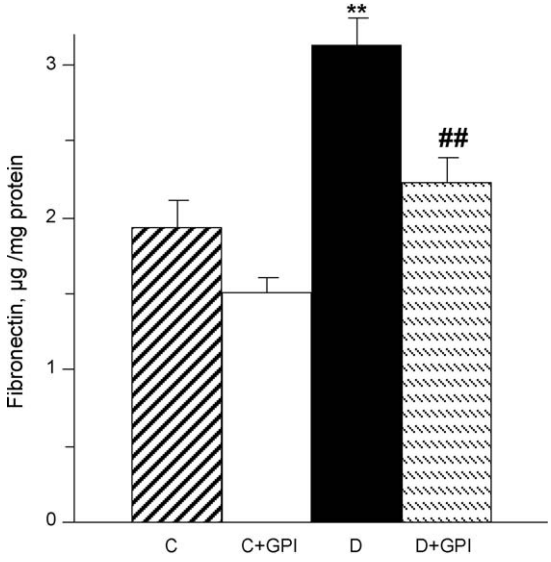

Fig. 4. PARP inhibition essentially prevents diabetes-induced renal cortex fibronectin accumulation. C – control group; D – diabetic group. Mean ± SEM; n = 7–10 per group. $^{**}p < 0.01$ vs controls; $^{\#\#}p < 0.01$ vs untreated diabetic group.

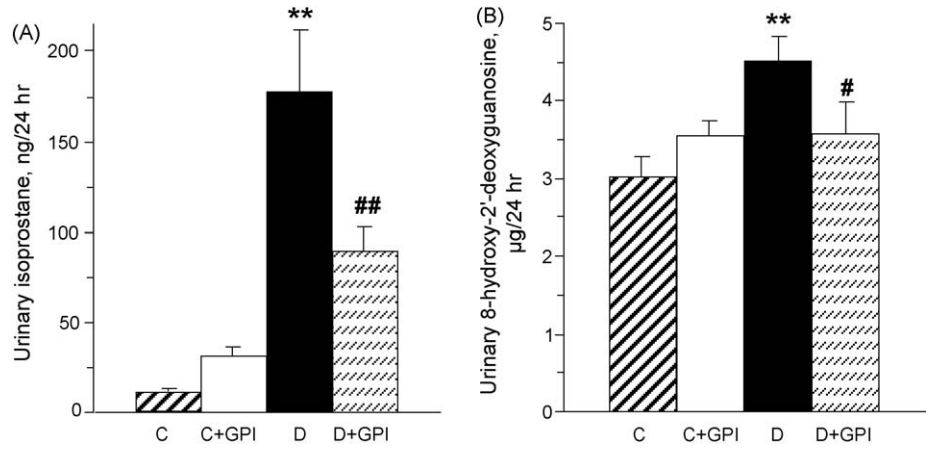

Fig. 5. PARP inhibition counteracts diabetes-induced urinary isoprostane (A) and 8-hydroxy-2′-deoxyguanosine (B) excretion. C – control group; D – diabetic group. Mean ± SEM, $n = 8–10$ per group. $^{**}p < 0.01$ vs controls; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with untreated diabetic group.

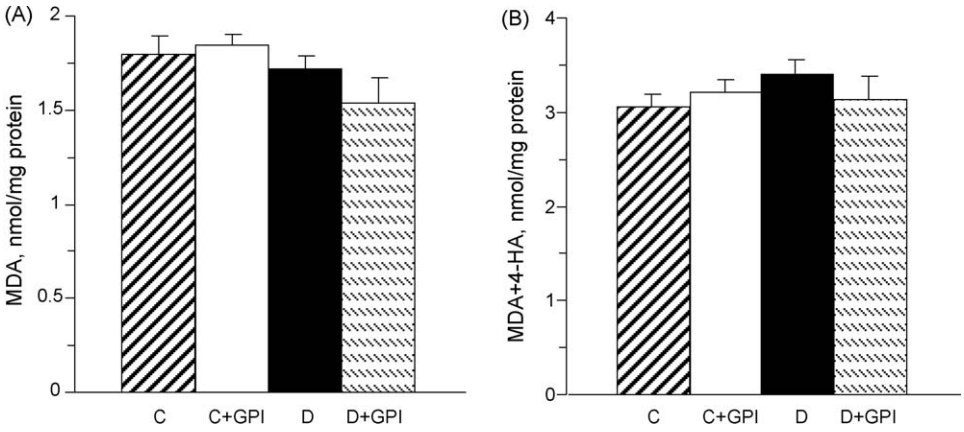

Fig. 6. Neither diabetes nor PARP inhibition affects renal malondialdehyde (A) or malondialdehyde plus 4-hydroxyalkenal (B) concentrations in control or diabetic rats. C – control group; D – diabetic group. Mean ± SEM, $n = 6–10$. $^{*}p < 0.05$ vs controls.

diabetic rats compared with controls, consistent with activation of the whole body lipid peroxidation and increased oxidative damage to DNA. GPI-15,427 treatment reduced urinary isoprostane and 8-hydroxy-2′-deoxyguanosine excretion in diabetic rats, without significantly affecting this variable in the non-diabetic controls.

Renal MDA and MDA plus 4-HA concentrations were indistinguishable among control and diabetic rats maintained with or without GPI-15,427 treatment (Fig. 6A and B).

Renal NT concentration was increased 1.3-fold in diabetic rats compared with controls, and this increase was not affected by a PARP inhibitor treatment (Fig. 7A). iCAM-1 concentrations were

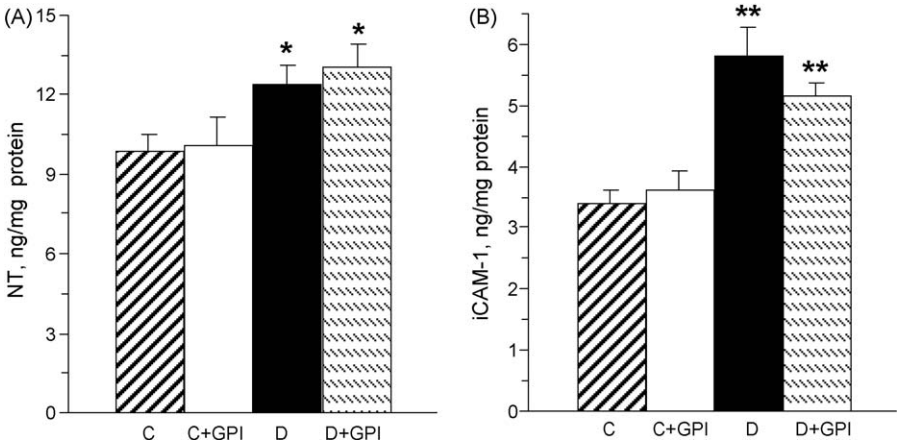

Fig. 7. PARP inhibition does not affect diabetes-induced renal cortex nitrotyrosine (A) or soluble intercellular adhesion molecule-1 (B) accumulations. C – control group; D – diabetic group. Mean ± SEM, $n = 7–10$. $^{**}p < 0.01$ compared with controls.

increased 1.7-fold in the renal cortex of diabetic rats compared with controls, and this increase was not significantly affected by a PARP inhibitor treatment (Fig. 7B).

4. Discussion

The findings described herein provide the first evidence of the important role for PARP activation in multiple manifestations of kidney disease associated with long-term Type 1 diabetes. PARP activation manifest by accumulation of poly(ADP-ribosyl)ated proteins is clearly manifest in the renal cortex of rats with 28-week duration of STZ-diabetes, and triggers multiple mechanisms implicated in diabetic nephropathy.

Albuminuria is a hallmark of diabetic kidney disease and an important predictor of a decline in renal function [37]. Understanding the pathogenesis of urinary albumin excretion in diabetic nephropathy is important to improve methods for early diagnosis and treatment. Multiple pathogenetic mechanisms including, but not limited by, non-enzymatic glycation/glyocoxidation [9,10], activation of angiotensin-converting enzyme [9,38], aldose reductase [7,8,39], protein kinase C [11,40], and 12/15-lipoxygenase [12,41], mesangial ion channel dysregulation [42], growth factor imbalances [43–46], impaired fatty acid metabolism [47], and oxidative stress [17,48–50] have been implicated in albuminuria associated with diabetes. Recently, we reported that PARP activation plays an important role in albuminuria associated with early nephropathy in STZ-diabetic rats [35]. The results of the present study, consistent with findings of others in the leptin receptor-deficient (*db*/*db*) mouse model of Type 2 diabetes [34], suggest that PAR activation is involved in albuminuria in kidney disease in chronic diabetes. A partial inhibition of diabetes-associated PARP activation in the renal cortex with GPI-15,427, 30 mg kg^{-1} d^{-1}, was associated with alleviation of albuminuria and blunting of the elevation of the urinary albumin/creatinine ratio.

Several growth factors and, among them, TGF-β, VEGF, pigment epithelium-derived factor, connective tissue growth factor, insulin-like growth factor, and basic fibroblast growth factor have been implicated in the pathogenesis of diabetic nephropathy [43–46,51,52]. TGF-β and VEGF accumulate in the kidney at both early [35,48] and advanced (the present study) stages of diabetes. TGF-β, through its Smad3 signaling pathway, is involved in diabetes-associated accumulation of mesangial extracellular matrix [24]. The important role for TGF-β in diabetic kidney disease is supported by studies with neutralizing anti-TGF-β antibodies, antisense TGF-β oligodeoxynucleotides, and Smad3 knockout mice in which renal TGF-β binding, knockdown, or knockout prevented or even reversed renal hypertrophic and profibrotic changes in diabetic mice [24]. VEGF, another important player in diabetic kidney disease, has been implicated in renal hyperfiltration, albuminuria, and glomerular hypertrophy in animal models of both Type 1 [44] and Type 2 [53,54] diabetes. A neutralizing VEGF antibody was found effective against the afore-mentioned changes [44,53,54]. We recently found that PARP inhibition counteracted renal VEGF accumulation in early diabetes [35], consistent with previous findings of our group and others implicating PARP activation in diabetes- and hypoxia-induced retinal VEGF formation [55] as well as angiogenesis [56,57]. The present study revealed that an effect of the PARP inhibitor GPI-15,427 on renal VEGF accumulation persists with prolongation of diabetes indicative of the important role for PARP activation in upregulation of VEGF associated with advanced diabetic nephropathy.

Fibronectin is a high-molecular weight extracellular matrix glycoprotein that binds to membrane-spanning receptor proteins, integrins. In addition to integrins, fibronectin also binds to extracellular matrix components such as collagen, fibrin and heparan sulfate proteoglycans (e.g. syndecans). Several factors in the diabetic milieu including a highly reactive dicarbonyl compound methylglyoxal [58], superoxide [59], nitric oxide [60], activated Ras (GTP-binding proteins of small-molecular-weight) [59], and increased aldose reductase activity [61,62] have been reported to increase fibronectin expression in renal mesangial and proximal tubular cells. Renal fibronectin accumulation has been identified in several animal models of diabetic nephropathy, and has been found amenable to correction by pharmacological agents counteracting expansion of the mesangial extracellular matrix [9,62]. The non-specific PARP inhibitor 3-aminobenzamide has been reported to reduce fibronectin mRNA expression and to prevent mesangial expansion in STZ-diabetic rats [63]. However, the study does not contain quantitation of the mesangial expansion data and, furthermore, evidence of a robust inhibition of renal poly(ADP-ribosyl)ation by 3-aminobenzamide. In our previous study, fibronectin concentration was increased in the kidney of rats with 3-month duration of STZ-diabetes, and this increase was counteracted, although not completely prevented by two structurally unrelated PARP inhibitors, 1,5-isoquinolinediol and GPI-15,427 [35]. The present findings suggest that inhibitory effect of GPI-15,427 on renal fibronectin accumulation persists in the chronic STZ-diabetic model. This provides the rationale for further, more detailed, evaluation of PARP inhibitors and PARP inhibitor-containing drug combinations as potential new therapies for the prevention of mesangial expansion and resulting obliteration of capillary lumen, glomerulosclerosis, and the end-stage renal disease often associated with advanced Type 1 diabetic nephropathy.

Growing evidence suggests that the relationship between diabetes-induced oxidative-nitrosative stress and PARP activation is bi-, rather than unidirectional, i.e., reactive oxygen (ROS) and nitrogen species induce PARP activation, whereas PARP activation, in turn, leads to oxidative damage. Such relationship between the two phenomena has been demonstrated for several tissue-sites for diabetic complications [9,34,35,64]. In particular, we reported that PARP activation contributes to systemic oxidative stress manifest by increased urinary isoprostane and 8-hydroxy-2′-deoxyguanosine excretions as well as renal oxidative-nitrosative stress manifest by elevated renal MDA plus HA and NT concentrations [35]. Others [34] implicated PARP activation in ROS generation in high glucose-exposed cultured glomerular podocytes. The present findings indicating that GPI-15,427 counteracts increases in urinary isoprostane and 8-hydroxy-2′-deoxyguanosine excretion, but not renal NT accumulation, in rats with 28-month duration of STZ-diabetes, may suggest that whereas PARP activation plays an important role in the whole body oxidative damage to lipids and DNA, its role in renal peroxynitrite formation in long-term Type 1 diabetes is minor. Note, however, that GPI-15,427, at the dose of 30 mg kg^{-1} d^{-1}, only partially inhibited renal poly(ADP-ribosyl)ated protein overexpression in the chronic STZ-diabetic model. Evidently, further studies with higher doses of PARP inhibitors that would result in a robust inhibition of poly(ADP-ribosyl)ation, are required to sort out the relationship between nitrosative stress and PARP activation in advanced diabetic nephropathy. In contrast to our previous studies in rats with shorter (4 weeks and 12 weeks [35,65]) durations of diabetes, no differences in renal lipid peroxidation product accumulation were found between control and 28-week STZ-diabetic rats treated with or without GPI-15,427. A potential explanation may be related to a protracted treatment with suboptimal doses of insulin, as insulin reduces renal lipid peroxidation product accumulation in chronic diabetes [50]. Renal iCAM-1 concentration was increased in diabetic rats, consistent with low-grade inflammation recently implicated in the pathogenesis of diabetic nephropathy [66,67] as well as other diabetic

complications. This inflammatory marker was not affected by a PARP inhibitor treatment. Note, that other studies demonstrated that (1) PARP inhibition suppressed adhesion molecule (iCAM-1, vascular cell adhesion molecule-1, E-selectin) overexpression in intermittent high glucose-exposed endothelial cells [68]; and (2) PARP gene deficiency protected from lipopolysaccharide-induced iCAM-1 overexpression in glial cells [28]. Apparently, further studies are needed to determine whether renal cortex iCAM-1 expression would be suppressed under conditions of a more robust PARP inhibition, or mechanisms, other than PARP activation, play a major role in renal iCAM-1 accumulation in chronic Type 1 diabetes.

In conclusion, PARP activation plays an important role in multiple manifestations of kidney disease associated with long-term insulin-dependent diabetes. These findings provide rationale for development and further studies of PARP inhibitors and PARP inhibitor-containing combination therapies, for prevention and treatment of diabetic nephropathy.

Acknowledgments

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