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Original Article

Poly(ADP-ribose) polymerase inhibition restores bladder function by suppressing bladder apoptosis in diabetic rats

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Abstract: Although diabetic cystopathy has a higher prevalence in patients with diabetes mellitus, its mechanism is still unclear and there is no effective therapeutic strategy. Previously, we have found an association between diabetic cystopathy and oxidative stress-induced bladder apoptosis, which results from activation of poly(ADP-ribose) polymerase (PARP) pathway-induced imbalance in Bcl-2 family proteins. In the present study, diabetic rats were treated with nicotinamide (a PARP inhibitor). Then, the bladder function, apoptosis of bladder, molecular changes of Akt-driven survival pathway, concentrations of malondialdehyde (MDA), ATP, nicotinamide adenine dinucleotide (NAD+) were assessed. When compared with the normal control group, untreated diabetic group showed impairment of bladder function, increased PARP activity and bladder apoptosis. In addition, the expression of phospho-Akt, phospho-Bad and Bcl-2, and concentrations of ATP and NAD+ significantly decreased, whereas concentrations of MDA, expression of Bax, amount of cytochrome *c* in cytoplasm and activity of caspase-3 and caspase-9 significantly increased. Treatment with nicotinamide significantly recovered these molecular and histological alterations, and restored bladder function. These results indicate that over-activation of PARP pathway in diabetic rats enhances bladder apoptosis via energy failure, inactivation of Akt-driven survival pathway and activating mitochondrial apoptotic pathway resulting in bladder dysfunction, which could be prevented by treatment with nicotinamide. These findings may be applied to develop novel therapeutic target for the prevention and treatment of diabetic cystopathy.

Keywords: Apoptosis, diabetes mellitus, diabetic cystopathy, poly(ADP-ribose) polymerase, protein kinase B

Introduction

Diabetic cystopathy, a form of bladder dysfunction, has a higher prevalence ranging from 25% to 87% in patients with diabetes mellitus [1], and causes important secondary complications, such as urinary tract infection, vesicoureteral reflux and hydroureteronephrosis, pyelonephritis, nephrolithiasis [2]. According to the traditional theory, mechanism of diabetic cystopathy is multi-factorial including alterations in the detrusor smooth muscle function, urothelial dysfunction and changes in the innervation or function of the neuronal components [3]. However, due to the mechanism is still unclear, there is no effective therapeutic strategy for diabetic cystopathy and remains to be elucidated extensively [4].

Increasing evidence has established an association between diabetic cystopathy and oxidative stress-induced bladder apoptosis. Studies from experimental animal models have found that oxidative stress and the number of apoptotic cells within smooth muscle and urothelial cell layers significantly increase in diabetic bladder compared to the control bladder [4-9]. In addition, report based on ultra-structural morphological study indicated that onset of bladder apoptosis is dependent on course of diabetes [10]. Moreover, therapeutic using vitamin E or urine derived stem cells significantly suppresses oxidative stress and reduces bladder apoptosis [5, 9]. These favorable results prompt that increasing oxidative stress and subsequent induction of apoptosis may as a mechanism plays an important role in the development diabetic cystopathy.

Oxidative stress is a major etiologic factor in the pathogenesis of diabetic complications in

both types of diabetes mellitus [11]. Oxidative stress is often defined as an imbalance between free radical productions and antioxidant capability. Hyperglycemia induces overproduction of advanced glycation end products and free radicals, e.g. reactive oxygen species, reactive nitrogen species, that directly oxidize and damage DNA, proteins and lipids, leading to potential tissue damage [12]. Moreover, increase in oxidative stress also subsequently cause inactivation of protein kinase B (Akt) and activation of poly(ADP-ribose) polymerase (PARP) pathways, contributing to the development of diabetic complications by inducing apoptosis or cell death [13-16]. PARP is an abundant nuclear enzyme of eukaryotic cells, which is activated by DNA breaks and plays a key role in a wide range of physiological cellular functions, including DNA repair, gene transcription and maintenance of genomic stability, apoptosis and cell death [14, 15]. It has been demonstrated that genetic disruption or pharmacologic inhibition of PARP have beneficial effects on the prevention of diabetic complications including neuropathy, retinopathy, nephropathy and cardiomyopathy [14-17], suggesting that inhibition of PARP may be a useful therapeutic approach for treatment of diabetic complications.

Previously, we have found that diabetic bladder apoptosis results from activation of PARP pathway-induced imbalance in Bcl-2 family proteins via activation of the c-jun-N-terminal kinase (JNK) and nuclear factor-κB (NF-κB) signaling pathways [18, 19]. However, the mechanisms of PARP-induced diabetic bladder apoptosis are not completely understood. Evidence has indicate that activation of Akt, well known upstream of Bcl-2 family proteins, plays a pivotal role in suppression of PARP-induced apoptosis [16, 20]. Therefore, the aim of this study was to investigate whether PARP-induced inactivation of Akt-driven survival pathway is involved in diabetic cystopathy and bladder apoptosis.

Materials and methods

Experimental animals

Eight-week-old male Sprague-Dawley rats were randomly divided into 3 groups (n=8 in each group): normal control group, diabetic group (DM) and diabetic treated with a PARP inhibitor, nicotinamide (vitamin B3), (DM+Vit-B3). Diabetes was induced by intraperitoneal injection of 50 mg/Kg streptozotocin (STZ, Sigma-Aldrich, St Louis, MO, USA), and the control group was treated with vehicle (0.1 M, ph 4.5 citrate buffer solution). Three days after STZ injection, the rat was considered to be diabetic when the blood glucose level was higher than 16.7 mmol/l (300 mg/dl). Eight weeks after induction of diabetes, the DM+Vit-B3 group was treated with nicotinamide (400 mg/kg/day, i.p.) for 4 weeks. This dose was selected because it has been previously demonstrated that treatment with a 400 mg/kg dose of nicotinamide exerts protective effects on diabetic cystopathy and is well-tolerated with no side effects [19]. This study was approved by the Animal Ethics Committee of Ninth People's Hospital, School of Medicine, Shanghai Jiaotong University. Animal welfare and experimental procedures were strictly in accordance with the guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the related ethics regulations of Ninth People's Hospital, School of Medicine, Shanghai Jiaotong University.

Conscious cystometry

To evaluate bladder function, bladder catheter implantation was performed twelve weeks after induction of diabetes. Four days after the catheter implantation, cystometry was performed as previously described [19]. The following urodynamic parameters were measured and analyzed by a computerized system (PowerLab, AD Instruments, Colorado Spring, CO, USA): basal pressure, micturition interval, voiding volume, peak micturition pressure. The residual urine volume of normal control rats was calculated by subtracting voiding volume from the infused saline volume. In contrast, the bladders of diabetic rats were immediately exposed through lower midline abdominal incisions after completion of the functional study, and the residual urine was collected by using a syringe since indirect calculation of residual urine volume in diabetic rats is inappropriate [19]. Subsequently, the rats were killed and the bladders were extirpated. The bladder dome was removed and part of remaining bladder tissue was maintained overnight in a 10% formaldehyde solution, then paraffin embedded for histological studies. The rest of the tissue was rapidly frozen in liquid nitrogen and stored at -80°C until processing.

Table 1. Changes in body weight, bladder wet weight and blood glucose, levels of MDA, ATP and NAD+

Mean variable	Group		
	Control	DM	DM+Vit-B3
General features			
Initial body weight (g)	312.24 ± 24.43	320.99 ± 11.94	321.13 ± 8.29
Final body weight (g)	629.13 ± 18.41	379.10 ± 38.32‡	397.7 ± 35.86‡
Final blood glucose (mmol/L)	5.49 ± 0.30	27.58 ± 4.19‡	26.46 ± 4.03‡
Bladder wet weight (mg)	140.40 ± 21.95	296.74 ± 27.09‡	299.06 ± 67.98‡
Bladder wet weight/Body weight (mg/g)	0.22 ± 0.04	0.79 ± 0.13‡	0.76 ± 0.20‡
Measurement of MDA, ATP and NAD+			
Concentrations of MDA (nmol MDA/mg protein)	3.40 ± 0.39	10.40 ± 1.62‡	4.18 ± 0.56†,**
Concentrations of ATP (nmol ATP/mg protein)	10.62 ± 1.21	3.90 ± 0.61‡	9.54 ± 1.0**
Concentrations of NAD+ (pmol NAD+/mg protein)	174.30 ± 19.85	73.22 ± 11.42‡	162.75 ± 14.82**

Data are means ± SD (n=8 in each group). †$P < 0.05$, ‡$P < 0.01$ vs. control group; **$P < 0.01$ vs. DM group. Control: normal control group; DM: diabetic group; DM+Vit-B3: diabetic group treated with PARP inhibitor nicotinamide (vitamin B3). MDA: malondialdehyde; NAD+: nicotinamide adenine dinucleotide.

Detection of apoptosis

Bladder apoptosis was assessed by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) method, using an ApopTag peroxidase in situ Apoptosis Detection Kit (Chemicon International, Temecula, CA, USA) as previously described [19]. For quantitative analysis of the degree of apoptosis, 5 high-power (×400) fields in each slide were randomly selected and the apoptotic index was expressed as the percentage of apoptotic cells relative to the number of total cells in a given area. The image analysis was performed in a blinded fashion and the same standards in all groups.

Measurement of malondialdehyde, ATP and nicotinamide adenine dinucleotide

The levels of malondialdehyde (MDA), ATP and nicotinamide adenine dinucleotide (NAD+) in the bladder were determined using a Bioxytech MDA-586 Assay Kit (Oxis Research, Portland, Oregon, USA), ATP Colorimetric/Fluorometric Assay Kit (BioVision, Mountain View, CA, USA) and NAD+/NADH Quantification Kit (BioVision, Mountain View, CA, USA) according to the manufacturer's instructions, respectively. The levels of MDA, ATP, and NAD+ were normalized based on the protein concentration in the tissue.

Western blot analysis

After the bladder tissues were homogenized in an ice-cold lysis buffer (pH 7.2) containing 0.3 M sucrose, 200 mM HEPES, 1 mM EDTA, protease inhibitor cocktail (Sigma, St Louis, MO, USA), and phosphatase inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA), whole-cell homogenates was centrifuged at 14,000 g at 4°C for 20 minutes and supernatants were collected. Equal amounts of proteins (20-50 μg) were electrophoresed on SDS-polyacrylamide gel, and incubated overnight at 4°C with primary antibodies i.e., anti-poly(ADP-ribose) (PAR; 1:1,000, Trevigen, Gaithersburg, MD, USA), anti-Akt and anti-phospho-Akt at Ser-473, anti-Bad, anti-phospho-Bad at Ser136 (1:1,000, respectively; Cell Signaling Technology, Beverly, MA, USA), Bcl-2 and Bax (1:1,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membranes were then incubated with appropriate horseradish peroxidase-linked secondary antibodies (Pierce Biotechnology, Rockford, IL, USA) for 2 hours at room temperature and visualized using the ECL detection system (Pierce Biotechnology, Rockford, IL, USA). The results of Western blots were quantified by densitometry using Bio-Rad imaging software Quantity One 4.6.2 (Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of cytochrome c, caspase-3 and caspase-9

The amount of cytochrome *c* in cytoplasm and activity of caspase-3 and caspase-9 in bladder tissue were determined by using cytochrome *c* ELISA kit (Abcam, Cambridge, UK), caspase-3 and caspase-9 colorimetric assay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions, respectively. The amount of cytochrome *c* and activity of cas-

Table 2. Awake cystometrography parameters

Mean variable	Group		
	Control	DM	DM+Vit-B3
Basal pressure (cmH_2O)	7.03 ± 2.48	7.76 ± 1.91	7.31 ± 2.25
Peak micturition pressure (cmH_2O)	52.97 ± 10.80	50.98 ± 11.38	56.81 ± 10.53
Micturition interval (s)	505.06 ± 70.93	1469.33 ± 217.81‡	825.47 ± 114.14‡,**
Voiding volume (ml)	1.33 ± 0.17	5.30 ± 1.87‡	3.10 ± 0.48‡,**
Residual urine volume (ml)	0.06 ± 0.02	1.59 ± 0.23‡	0.60 ± 0.19‡,**

Data are means ± SD (n=8 in each group). ‡$P < 0.01$ vs. control group; **$P < 0.01$ vs. DM group. Control: normal control group; DM: diabetic group; DM+Vit-B3: diabetic group treated with PARP inhibitor nicotinamide (vitamin B3). Basal pressure: the lowest average bladder pressure during filling; Peak micturition pressure: the maximum pressure during micturition; Micturition interval: time between the start to the end of one voiding cycle-at the beginning resting pressure to the end of the micturition phase; Voiding volume: the volume of expelled urine during micturition; Residual urine volume: postvoid residual urine volume.

pase-3 and caspase-9 were expressed as fold-change compared to the control.

Statistical analysis

All results are expressed as mean ± SD. Due to the modest sample size, a nonparametric Kruskal-Wallis test was performed to analyze the entire group and the Mann-Whitney U-test was used to compare between two groups. A P value < 0.05 was considered statistically significant.

Results

General features

Diabetic rats showed significant increase in blood glucose levels, absolute and relative bladder weights, and progressive loss of body weights compared with control rats. Treatment with nicotinamide did not affect blood glucose levels, body weights and bladder weights in diabetic rats (**Table 1**).

Treatment with nicotinamide improves bladder function

As shown in **Table 2** and **Figure 1**, conscious cystometry study showed that the mean micturition interval, mean voiding volume, and residual urine volume in the DM group significantly increased compared with the control group. These increases significantly reduced after treatment with nicotinamide, although the parameters did not recover to the level of the control group. However, there were no statistically significant differences in the resting, threshold, and peak micturition pressures among the three groups. These results suggest that activation of PARP may play an essential role in the impairment of bladder function in diabetic rats.

Treatment with nicotinamide reduces apoptosis in diabetic rat bladder

The number of TUNEL positive cells in the DM group was significantly higher than that in the control group. The increase in the number of apoptotic cells was significantly inhibited by treatment with nicotinamide (**Figure 2**). These results indicate that activation of PARP increases apoptosis in diabetic rat bladder.

Treatment with nicotinamide has antioxidant capacity and attenuates depletion of intracellular NAD+ and ATP pools in diabetic rat bladder

When compared to the control group, the DM group showed significant increases in MDA concentrations. Treatment with nicotinamide significantly reduced MDA concentrations (**Table 1**). These results indicate that treatment with nicotinamide have antioxidant capacity in diabetic rat bladder. In addition, compared to the levels in the control group, the DM group showed significantly lower ATP and NAD+ concentrations; whereas their concentrations were significantly increased in the DM+Vit-B3 group (**Table 1**). These results indicate that activation of PARP could deplete ATP and NAD+ in diabetic rat bladder.

Treatment with nicotinamide reduces amount of PAR and activates Akt pathway in diabetic rat bladder

Western blot revealed that PAR expression was significantly increased in the DM group compared to that in the control group. Treatment with nicotinamide significantly reduced the amount of PAR compared to that in the DM group (**Figure 3A** and **3B**). DM group showed

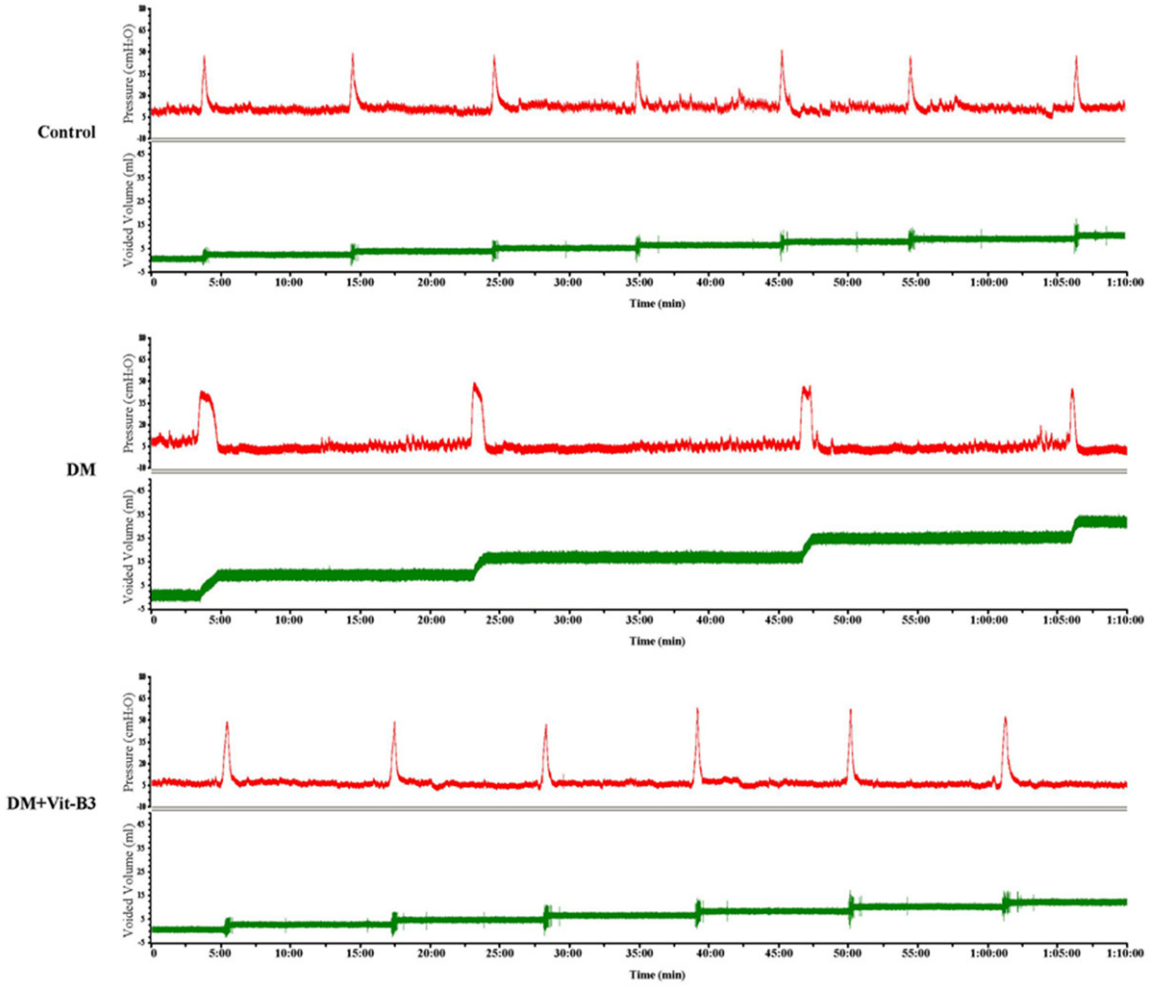

Figure 1. Awake cystometrography. Representative traces in normal rats (control) and diabetic rats treated without (DM) and with nicotinamide (DM+Vit-B3) 12 weeks after diabetes induction.

significantly lower levels of Akt and Bad phosphorylation compared to those in the control group, whereas their levels were significantly increased by nicotinamide treatment (**Figure 3A** and **3C**). In addition, the DM group expressed a significant decrease in Bcl-2 and a significant increase in Bax; as a result, the ratio of Bcl-2/Bax was significantly decreased. After treatment with nicotinamide, the ratio of Bcl-2/Bax significantly increased (**Figure 3A** and **3D**). These results indicate that activation of PARP induces inactivation of Akt and imbalance in Bcl-2 family proteins.

Treatment with nicotinamide reduces amount of cytochrome c and activity of caspase-3 and caspase-9

Amount of cytochrome *c* in cytoplasm and activation of caspase-3 and caspase-9 were significantly increased in the DM group compared to the control group. Treatment with nicotinamide significantly reduced the amount of cytochrome c in cytoplasm and activation of caspase-3 and caspase-9 compared to that in the DM group, but the levels were still higher than the control group (**Figure 4**). These results indicate that treatment with nicotinamide could attenuate release of cytochrome c from the mitochondria and caspase activity.

Discussion

Apoptosis or programmed cell death is a critical process in maintaining the structural integrity and homeostasis of organisms, which is characterized by various morphologic and biochemical changes of the cell, such as chromatin condensation, DNA fragmentation, alterations of mitochondrial membrane potential, activation

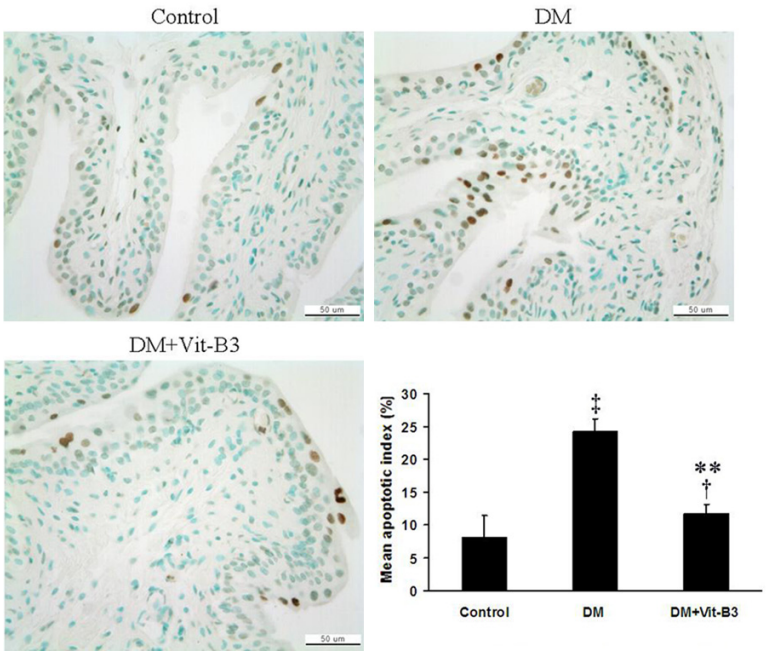

Figure 2. Detection of cell apoptosis. Representative micrographs show apoptotic cells as black-brown dots in TUNEL staining (magnification ×400). Bar graphs represent quantitative image analysis. Apoptotic index was defined as the percentage of apoptotic cells within the total number of cells in a given area. †P < 0.05, ‡P < 0.01 vs. the control group; **P < 0.01 vs. the untreated diabetic group. DM = diabetes mellitus; Vit-B3 = nicotinamide.

of caspases, formation of apoptotic bodies and loss of cell volume [21]. Integrity of autonomic innervation, cellular structure and metabolism of bladder is a prerequisite for normal bladder function [22], and several studies indicate a link between diabetic cystopathy and apoptosis [8-10]. Recently we found a mechanism of diabetes induced bladder dysfunction results from apoptosis through the activation of PARP pathway-induced imbalance in Bcl-2 family proteins in bladder tissue [18, 19]. The results from our previous studies and current study indicate that activation of PARP pathway induces apoptosis in diabetic rat bladder through several mechanisms.

Firstly, activation of PARP seems to induce bladder apoptosis via depletion of intracellular NAD+ and ATP pools. According to the classical view, the fundamental mechanism of PARP-induced apoptosis is the onset of intracellular energy depletion. Activation of PARP by DNA breaks is necessary for DNA repair when DNA damage is mild. However, in many excessive oxidative stress-related pathophysiological processes such as stroke, ischemia/reperfusion injury and diabetes, PARP can over-activate and subsequently induce depletion of intracellular NAD+ ATP pools, contributing to cell dysfunction or death [15]. In our study, increase in bladder apoptosis was associated with decreased NAD+ and ATP levels. After treatment with nicotinamide, the concentrations of NAD+ and ATP significantly increased. As a result, bladder apoptosis was reduced. These results indicate that the hyperglycemia induces over-activation of PARP, which cause bladder apoptosis via energy depletion.

In addition to the energy depletion, PARP-induced apoptosis in diabetic rat bladder is due to the imbalance in the expression of Bcl-2 family proteins. In the numerous mechanisms involved in apoptosis, the Bcl-2 family proteins are believed to play critical roles through regulation of mitochondrial membrane permeabilization, the pivotal event in the progression of apoptosis. Upon receiving the apoptotic stimulation, the pro-apoptotic members (e.g., Bax, Bak) bind to the outer mitochondrial membrane promote release of cytochrome *c* and apoptosis inducing factor (AIF) that activate the caspase-dependent and caspase-independent apoptotic pathways. In contrast, the anti-apoptotic members (e.g., Bcl-2, Bcl-xl) of Bcl-2 family proteins prevent release of the mitochondrial internal content [21]. Therefore imbalance in the expression of pro- and anti-apoptotic Bcl-2 family proteins is a critical determinant of apoptosis, especially the Bax/Bcl-2 ratio [23, 24]. In addition, Bad, a pro-apoptotic member of the Bcl-2 family proteins is also involved in onset of apoptosis. Upon receiving the apoptotic stimulation, bad translocates from cytosol to mitochondria, where it binds to and inhibits Bcl-2 and Bcl-xl, and thus allowing Bax/Bak-triggered apoptosis. In contrast, phosphorylation of Bad on Ser 112 or 136 by anti-apoptotic kinases results its cytosolic localization as an inactive form and inhibits the interaction between Bad

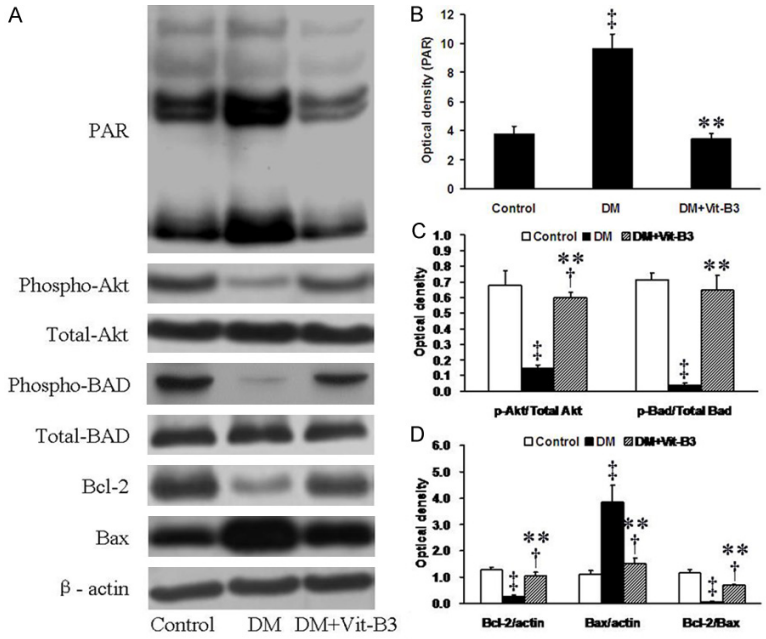

Figure 3. Western blot analysis. Representative western blot and bar graphs show the expression and densitometric ratios of PAR to β-actin (A and B), phosphorylated Akt to total Akt, phosphorylated Bad to total Bad (A and C), Bcl-2 to Bax, (A and D) in the control (lane 1), diabetic (lane 2) and nicotinamide treated (lane 3) groups, respectively. The results of western blots were quantified by densitometry using Bio-Rad imaging software Quantity One 4.6.2 (Bio-Rad Laboratories, Hercules, CA, USA). †P < 0.05, ‡P < 0.01 vs. the control group; **P < 0.01 vs. the untreated diabetic group. PAR = poly(ADP-ribose); Akt = protein kinase B; DM = diabetes mellitus; Vit-B3 = nicotinamide.

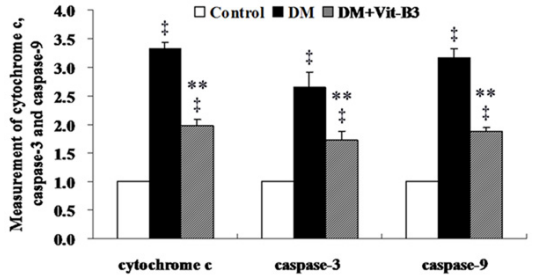

Figure 4. Measurement of cytochrome c, caspase-3 and caspase-9: amount of cytochrome c in cytoplasm and activity of caspase-3 and caspase-9 were evaluated by the ELISA based method and expressed as a fold-change compared to the control. ‡P < 0.01 vs. the control group; **P < 0.01 vs. the untreated diabetic group. DM = diabetes mellitus; Vit-B3 = nicotinamide.

and Bcl-XL to suppress Bax-triggered apoptosis [23, 24]. In the previous and present studies, significant decrease in phosphorylation of Bad and significant increase in Bax/Bcl-2 ratio, PARP activity and apoptosis were observed in diabetic rat bladder. After treatment with two specific PARP inhibitors, either 3-aminobenzamide (3-AB) or nicotinamide, these alterations were attenuated. In addition, in the previous and present studies we also found that the translocation of AIF from mitochondria to nucleus, amount of cytochrome *c* in cytoplasm and activation of caspase-3 and caspase-9 were parallel with the ratio of Bax/Bcl-2. These findings indicate that activation of PARP may cause aberrant Bcl-2 family proteins expression and subsequently activates mitochondrial apoptotic pathway leading to apoptosis in diabetic rat bladder.

Another involvement in PARP-induced diabetic rat bladder appears to be resulted from inactivation of Akt-driven survival pathway. In the present study, we found that decreased phosphorylation of Akt was parallel with increase in PARP activity and bladder apoptosis. Since intracellular levels of NAD+ did not affected by the inhibition of Akt [25], Akt pathway may play an important role as an energy independent modulator in PARP-induced diabetic bladder apoptosis. In fact, activation of Akt plays a pivotal role in protecting the mitochondria by PARP inhibition [20], and the inhibition of PARP revealed cell survival by recovery of Akt activation in oxidative stress conditions, including hyperglycemia, ischemia/reperfusion and septic shock [16, 25-28]. Akt is a serine-threonine kinase that has a central role in modulation of prosurvival and antiapoptotic signaling, which can directly or indirectly phosphorylate pro-apoptotic molecules such as Bad, caspase-9 and NF-κB to inactivate them and promote cell survival [29, 30]. Furthermore, Akt can also prevent activation of the apoptotic JNK pathway by direct binding to JNK or indirect phosphorylation of apoptosis signal-regulating kinase-1 [30]. Moreover, we have previously found that activation of the JNK and NF-κB signaling pathways are involved in diabetic rat

bladder apoptosis [18, 19]. Taken together, these results indicate that activation of Akt-driven survival pathway may play a critical role in inhibition of PARP-induced apoptosis in diabetic rat bladder via suppression of apoptotic JNK and NF-κB pathways, although the exact mechanism by which the PARP pathway induces inactivation of Akt in diabetes has not been completely demonstrated. Yongzhi L et al have found that expression of transforming growth factor-beta (TGF-β) significantly increased in diabetic rat bladder [31]. Studies have shown that TGF-β expression directly controlled by PARP and plays an important role in induction of apoptosis by the inactivation of Akt [30, 32]. This evidence may represent a link between PARP inhibition and protection of Akt phosphorylation in the diabetic bladder. In addition, the overall relationship of Akt, JNK and NF-κB pathways is complex; further studies are needed to investigate the molecular and functional interactions among Akt, JNK and NF-κB signaling pathways and their relevance for PARP induced bladder apoptosis.

Although diabetic cystopathy is not life threatening, its prevalence is higher than that of widely recognized complications such as neuropathy and nephropathy, and significantly affects quality of life [4]. Standard insulin treatment for diabetes can delay the occurrence of diabetic cystopathy by improving peripheral neuropathy [33]; nevertheless peripheral neuropathy in the late phase is irreversible with insulin replacement [34]. In addition, the little known about the mechanisms of diabetic cystopathy limits the development of the best prevention and treatment methods [4]. Therefore, the clinical relevance of this study is of utmost importance. Our previous and present studies demonstrate that inhibition of PARP with two specific PARP inhibitors 3-AB or nicotinamide had a benefit effect in the improving bladder function via recovering of structural alterations of bladder. Taken together, we suggest that PARP pathway may be a potential therapeutic target for the prevention and treatment of diabetic cystopathy.

The main limitation of this study was the focus on PARP pathway induced bladder apoptosis in diabetic cystopathy; other potential mechanisms implicated in diabetic cystopathy, such as peripheral neuropathy [35] was not investigated. However, neurodegeneration is due to apoptotic cell death in the ganglia [34] and inhibition of PARP has protective effects on diabetic neuropathy [15]. Moreover, treatment with nicotinamide improves complications of diabetic peripheral neuropathy by acting as both a PARP inhibitor and antioxidant [36] which is also evidenced by the change of MDA concentration in the present study. Therefore, we speculate that PARP inhibitors, especially nicotinamide have benefit effect on improving diabetic peripheral neuropathy. Further studies are needed to clarify this issue. In addition, the STZ-induced diabetic rat model mimics type 1 diabetes, and further studies should investigate the role of PARP inhibition in type 2 diabetes, which is the predominant form of diabetes.

In conclusion, the results of the present study suggest that over-activation of PARP pathway is involved in the pathogenesis of diabetes-related bladder dysfunction by the enhancement of bladder apoptosis via energy depletion, suppression of Akt phosphorylation, and mitochondrial apoptotic pathway activation. These novel findings provide additional evidence that the PARP pathway might be a potential therapeutic target for the prevention and treatment of diabetic cystopathy.

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Disclosure of conflict of interest

None.

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