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Original Article

Renoprotective effect of total glucosides of paeony in STZ-induced diabetic rats: role of thioredoxin-interacting protein

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Abstract: Background: Total glucosides of paeony (TGP), a traditional Chinese medicine, plays a therapeutic role in experimental diabetic nephropathy. However, the relationships between TGP and thioredoxin-interacting protein (Txnip) have not been clearly identified. Objectives: Demonstrating the relationships between TGP and Txnip. Methods: Diabetic rats, induced with streptozotocin, were randomly divided into model group and model group treated with TGP. TGP was orally administered daily at a dose of 50, 100 and 200 mg/kg for 8 weeks. Thioredoxin(Trx) system is a kind of system which can protect against oxidative stress and maintain the balance of oxidation-reduction. Levels of Trx, Txnip, apoptosis signal-regulating kinase 1 (ASK1) and p-p38 Mitogen-activated protein kinase (p-p38 MAPK) were assessed by immunohistochemistry, western blot analysis and/or real-time polymerase chain reaction (PCR). Results: After 8-weeks of treatment, elevated 24 h urinary albumin excretion rate (UAER) was obviously decreased by TGP treatment dosed at 50, 100 and 200 mg/(kg.d). The rats in each group were euthanized and tested for the renal functional and pathological detection, as well as the expression detection of Trx, Txnip and ASK1 of kidney tissues. Western blot analysis and real-time PCR indicated that the protein and mRNA expression of Txnip were increased in the diabetic rat kidneys, and these were significantly decreased by TGP treatment (50, 100 and 200 mg/kg). Immunohistochemistry and real-time PCR indicated that the protein and mRNA expression of Trx were decreased in diabetic rat kidneys, and were significantly increased by TGP treatment at 50, 100 and 200 mg/kg. ASK1 and p-p38 MAPK protein determined by immunohistochemistry and/or Western blot analysis were markedly increased in control diabetic rats when compared with the normal rats, while treatment with TGP with 50, 100 and 200 mg/kg could decrease the expression. Conclusion: Renoprotection of TGP in diabetic rats possibly contributed to the inhibition of the expression of Txnip, ASK1, p-p38 MAPK and promoted the expression of Trx.

Keywords: Diabetic nephropathy, thioredoxin-interacting protein, thioredoxin, apoptosis signal-regulating kinase 1, total glucosides of paeony

Introduction

Diabetic nephropathy (DN) is one of the leading causes of end-stage renal disease which is a common chronic complication of diabetes mellitus. The involvement of various theories associated with diabetes can be considered in the initiation and progression of DN [1]. Among them, oxidative stress has been suggested to play a significant role in the pathogenesis of DN [2].

Oxidative stress has been shown to play an important role in the development and progression of diabetic nephropathy, and the formation of reactive oxygen species (ROS) is a direct consequence of hyperglycaemia. The thioredoxin (Trx) system, which is composed of Trx, thioredoxin reductase (TrxR), and nicotinamide adenine dinucleotide phosphate (NADPH), is a key antioxidant system in defense against oxidative stress through its disulfide reductase activity regulating protein dithiol/disulfide balance [3]. Dysregulation in this system has been associated with metabolic, cardiovascular, and malignant disorders [4]. Recent studies showed that Txnip was found to be up-regulated by high glucose in proimal tubular cells, mesangial cells and vascular smooth muscle cells. The expression of Txnip protein and mRNA was increased in kidneys from both diabetic rats and patients with DN, and Txnip mediated the glucose-

induced impairment of Trx activity in cultured kidney cells [5]. Apoptosis signal-regulating kinase 1 (ASK1) also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5) is a member of the MAP3K family, and therefore a part of the MAPK pathway. The N-terminal portion of ASK1 interacts with the reduced form of Trx [6]. Moreover, Trx reduction inhibits the activity of ASK1 and the subsequent ASK1-dependent apoptosis [7].

Traditional herbal medicines have been widely used for the treatment of diabetes and diabetic complications in Asian countries for many years. It is suggested they have a promising future in diabetic prevention and treatment due to integrated effects [8]. *Paeonia lactiflora Pall* is one of the most important crude drugs in traditional Chinese medicine, which has a long history of use for rheumatoid arthritis, systemic lupus erythematosus [9, 10]. Total glucosides of paeony (TGP) are active compounds extracted from the dried roots of *Paeonia lactiflora Pall*. The therapeutic effects include its anti-inflammatory, antioxidative and immunoregulatory activities [11]. TGP was also approved by the China Food and Drug Administration (CFDA) to enter the market as a disease-modifying drug since 1998.

We have previously reported that TGP has been shown to have a therapeutic role in experimental DN [11, 12]. The key role of antioxidative stress in this disease has been concerning, but to what extent the thioredoxin system plays in the progress of DN and the renoprotection of TGP in DN remains poorly defined. As such, our study provides a possible mechanism and therapeutic target of TGP for DN treatment.

Materials and methods

Drugs and reagents

TGP was extracted and isolated from the root of Paeonia lactiflora Pall by ethanol reflux, n-butanol extraction, and macroreticularabsorption resin chromatography. The extract was determined to contain 41.1% paeoniflorin by high-performance liquid chromatog-raphy fingerprinting analysis. Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, Mo, USA). The microalbumin assay kit was purchased from Exocell Inc. (Philadelphia, Pa., USA). Trx, Txnip, and GAPDH primers and probes were purchased from Shanghai Sangon company (Shanghai, China). Reverse transcriptase Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase and RNA enzyme inhibitor were obtained from Promega (Madison, USA). Trlquick Resgent was obtained from Invitrogen (California, USA). The following antibodies were used in this study: rabbit anti-Txnip, Trx, ASK1, and p-p38 MAPK polyclonal antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Nitrocellulose membrane was obtained from Amersham Life Science (Little Chalfont, UK). The chemiluminescence kit was purchased from Amersham Life Science (Little Chalfont, UK). The immunohistochemistry kit (PV-9000) was bought from Beijing Zhongshan Biotechnology Inc (Zhongshan, China).

Animals

Male Munich-Wistar rats weighing 180-200 g were purchased from the Experimental Animal Center of Anhui Medical University. Animals were housed in a 12 hour light/dark cycle room with the temperature at about 24 ± 1°C and humidity at about 60%. The rats received humane care and were allowed free access to standard laboratory chow and tap water. All experimental protocols were approved by the Animal Ethics Committees of the Faculty of Anhui Medical University, in accordance with "Principles of Laboratory Animal Care and Use in Research" (Ministry of Health, Beijing, China).

Experimental design

The rats were intraperitoneally injected with STZ diluted with citrate buffer (0.1 M, pH 4.0) at a dose of 65 mg/kg following overnight fasting after several days of adaptation. Two days later, the diabetic state was confirmed by measurement of tail blood glucose levels using a reflectance meter (One Touch II; Lifescan LTD, Jinan China). We measured the blood glucose levels twice a week. Blood glucose > 16.7 mmol/L was accepted as diabetes. Avoiding any intergroup differences in blood glucose levels, diabetic rats were randomly divided into four groups (n = 10 per group). A normal group of rats was also included. The normal and control diabetic groups were intragastrically administered 0.5% sodium carboxymethylcellulose (CMC-Na), while the other groups were intragastrically administered TGP (suspended in 0.5% CMC-Na) orally at a dose of 50, 100

and 200 mg/kg daily. The effects of TGP 50, 100 and 200 mg/kg concentrations on albuminuria in diabetic rats were proved to be effective in the preliminary experiments.

Blood sample and tissue collection

At eight weeks, body weight was measured. After being fasted for 12 h, rats were subsequently anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and were placed on a temperature-regulated table. The blood samples were collected immediately by catheterizing the right jugular artery. Blood glucose levels were determined with a glucose analyzer. The kidneys were perfused *in vivo* via the abdominal aorta with 100 ml of normal saline at 4°C. The left renal vein was punctured to permit the perfusate to drain. Both kidneys were removed. The whole left kidney cortices were stored in 10% formaldehyde solution for immunohistochemical experiments. The right kidneys were stored at -80°C for the subsequent assays.

Urinary albumin excretion

Rats were placed in metabolic cages for collecting urine over a 24 hour period for measurement of albumin concentration before the time of death. Urine samples from each rat were combined and measured. Aliquots of the supernatant were frozen at -80°C after centrifugation for subsequent analysis of albumin concentration. Urinary albumin concentration was measured by enzyme-linked immunoabsorbent assay using an anti-rat albumin antibody.

Immunohistochemistry

Immunoperoxidase staining for Trx and ASK1 was performed on 10% formalin-fixed paraffin sections (2 μm). 3% hydrogen peroxide was used to close endogenous peroxidase and antigen retrieval adopted the microwave hot repairing method [microwave oven heating in 0.1 M sodium citrate (pH 6.0) for 10 min]. Tissue sections were incubated with 10% normal goat serum for 10 min followed by an overnight incubation with anti-Trx (1:100) and anti-ASK1 antibody (1:100) at 4°C. The sections were washed in phosphate buffer saline (PBS) and incubated with the appropriate horseradish peroxidase-labeled goat anti-rabbit/mouse polyclonal antibody for 30 min at 37°C. After rinsing, the results were visualized using 3, 3-diaminobenzidine (DAB, Sigma) to produce a brown color and the sections were counterstained with hematoxylin. Immunostaining of Trx and ASK1 in glomeruli was evaluated using the following semiquantitative scale: 0 = diffuse, very weak or absent staining; 1 = staining involving less than 25%; 2 = staining involving 25% to 50%; 3 = staining involving 50% to 75% and 4 = staining involving 75% to 100% [12]. Immunostaining of Trx and ASK1 in tubulointerstitium was quantified using the Image-Pro Plus 6.0 image analysis system by evaluating the positively stained area of the sections and the whole view area under the same light intensity for microscopy [13]. We randomly selected five visions from each section under high magnification and calculated the ratio of the positively stained area and the whole view area. The mean was taken. All scoring was performed blind.

Western blot analysis

Protein extraction from the kidneys was performed as follows. Kidney samples were homogenized and lysed in Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, boiled, centrifuged, and the supernatant recovered. Using bovine serum albumin as a standard, the protein content was estimated by the dye binding assay of Bradford. Samples were resolved on SDS-PAGE and electrotransferred onto nitrocellulose membranes. The membranes were initially blocked with phosphate buffer saline (PBS) containing 10% non-fat dry milk for 2 h and then incubated with specific primary antibody overnight at 4°C. After the blots were washed, they were incubated with a Horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG for 1 h at 37°C. The bound secondary antibody was detected by enhanced chemiluminescence. As a loading control, housekeeping protein β-actin was used. Positive immunoreactive bands were quantified densitometrically (Leica Q500 IW image analysis system) and expressed as ratio of Txnip, ASK1 and p-p38 MAPK to β-actin in optical density units.

RNA extraction and real-time PCR

The RNA extraction and real time PCR were performed as previously reported. According to the manufacturer's protocol, total RNA was extracted from the kidneys using Trizol reagent. 1 μg RNA was reverse-transcribed into cDNA using RevertAid Premium First Strand

Table 1. Clinical and metabolic parameters in the five groups of rats

Group	Dose (mg/kg)	Blood glucose (mmol/L)	Kidney weight/body weight (g/100 gBW)	AER[a] (mg/24 h)
Normal		6.94 ± 1.64	0.30 ± 0.04	0.38 ×/÷ 1.3
Control diabetic		31.97 ± 4.19**	0.56 ± 0.05*	1.87 ×/÷ 1.1**
Diabetic + TGP	50	30.62 ± 4.35	0.52 ± 0.02	1.32 ×/÷ 1.1#
	100	32.05 ± 4.24	0.50 ± 0.06	1.15 ×/÷ 1.1#
	200	27.20 ± 4.25	0.50 ± 0.04	0.65 ×/÷ 1.1##

Data are expressed as means ± SEM. Number of rats in each group was 10. *$p < 0.05$, **$p < 0.01$ compared with normal group. #$p < 0.05$, ##$p < 0.01$ compared with control diabetic group. [a]Shown as geometric mean +/- tolerance factor.

cDNA Synthesis Kit (Fermentas, Burlington, Canada). The cDNA was amplified by real-time PCR with Synergy Brands (SYBR) Green PCR master mix kit (Invitrogen, California, USA) to determine the quantity of mRNA and the housekeeping gene GAPDH was used as the internal control. The SYBR Green assays were performed in triplicate on a 7500 real-time instrument (Applied Biosystems, CA, USA). The primers to detect mRNA were as follows: Txnip, 5'-TCAGTCAGAGGCAATCACATTA-3' and 5'-GGAGCCAGGGACACTAACATAG-3'; Trx, 5'-CCTTCTTTCATTCCCTCTGTGA-3' and 5'-CCCAACCTTTTGACCCTTTTTA-3'; GAPDH, 5'-ACAGCAACAGGGTGGTGGAC-3' and 5'-TTTGAGGGTGCAGCGAACTT-3'. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with values normalized to the reference gene Gyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical analysis

The measurement data were expressed as $\bar{x} \pm s$. The intergroup difference was compared using ANOVA, the paired comparison between two groups used Tukey test. Since urinary albumin excretion rate followed a skewed distribution, log transformation was performed prior to statistical analysis of this parameter. Statistical significance was set at $P < 0.05$.

Results

Effects of TGP on clinical and metabolic parameters in diabetic rats induced by STZ

Table 1 shows the effects of TGP on clinical parameters in STZ-induced diabetic rats. Rats in the control diabetic group had reduced body weight and increased blood glucose levels. However, rats with TGP treatment were observed to have no effects on body weight and blood glucose. The ratio ofkidneyweighttobodyweight was dramatically higher in the control diabetic group than that in normal group. Compared with diabetic control, the ratio of kidney weight to body weight was not decreased with TGP treatment for 8 weeks. Urinary albumin levels are a selective marker of glomerular injury and elevated rates of albumin excretion are a marker of progressive nephropathy. In the control diabetic group, albuminuria was significantly increased when compared to normal rats, where treatment with TGP dose-dependently attenuated albuminuria in the diabetic rats. ED50 value was 139.4 mg/kg.

Effects of TGP on Txnip expression in the kidney of diabetic rats induced by STZ

The effect of TGP on renal Txnip protein expression in diabetic rats induced by STZ. Densitometric analysis of the Western blot showed that the protein levels of Txnip were strikingly higher in the control diabetic rats than those in the normal rats. Compared with those in the control diabetic group, the expression of Txnip were significantly decreased by TGP administration with 50, 100 and 200 mg/kg (**Figure 1A**). The relative levels of Txnip mRNA in the control diabetic group was much higher than the normal group. The Txnip mRNA levels in the TGP treatment groups were strikingly decreased compared with those of the control diabetic group (**Figure 1B**).

Effects of TGP on Trx expression in the kidney of diabetic rats induced by STZ

Trx was observed in the glomerulus and tubulointerstitium. In the kidneys of normal rats, there was abundant staining for Trx. In contrast, minimal Trx was expressed in the kidneys of the diabetic rats. This low expression was intensified in diabetic rats treated with TGP (50, 100 and 200 mg/kg). These results indicated that treatment with TGP significantly increased the expression of Trx in the diabetic rats. (**Figure 2A** and **Table 2**). The relative levels of Trx mRNA in the control diabetic group was greatly lower than the normal group. The Trx mRNA levels

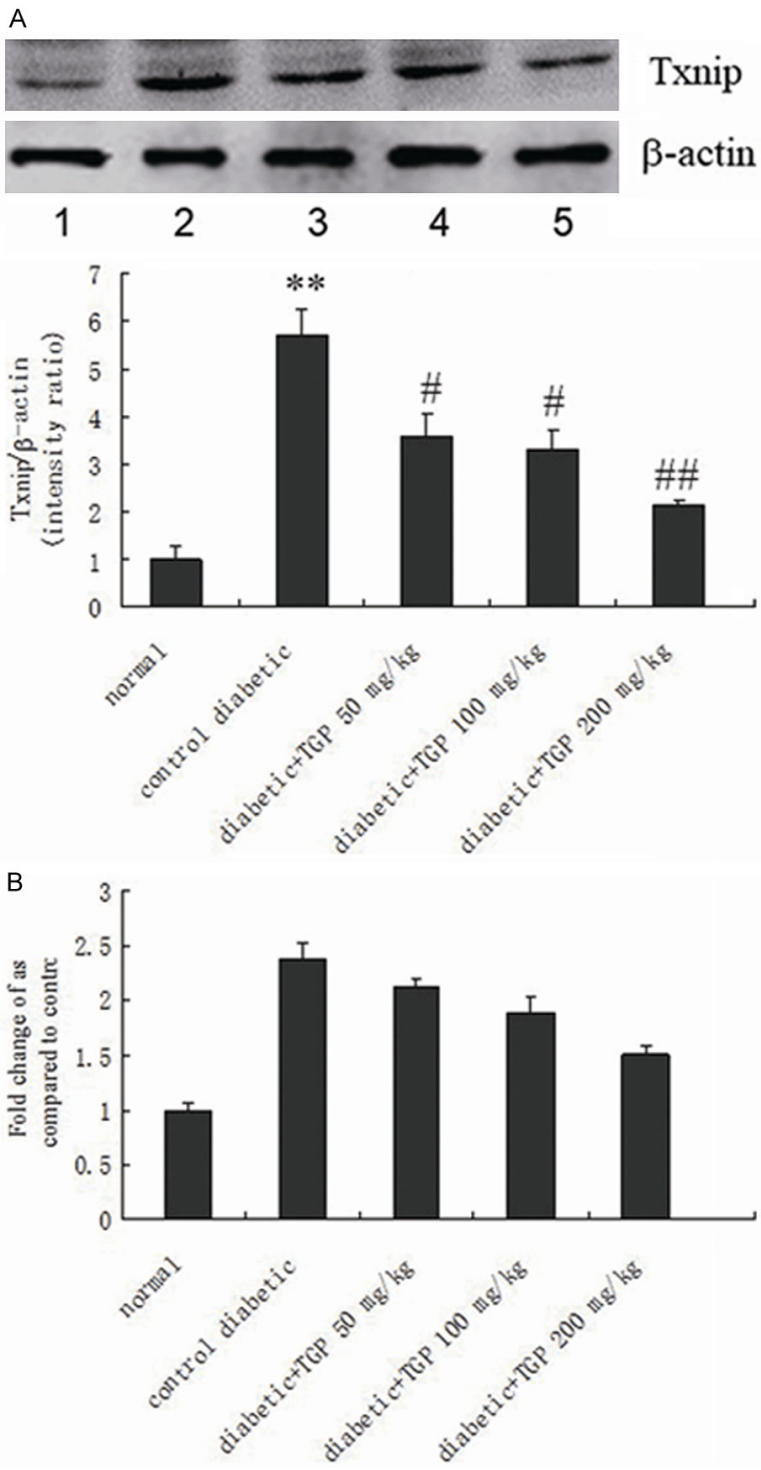

Figure 1. A. Western blot analysis of Txnip in renal tissue in five groups of rats. 1. normal; 2. control diabetic; 3. diabetic + TGP 50 mg/kg; 4. diabetic + TGP 100 mg/kg; 5. diabetic + TGP 200 mg/kg. $^{**}p < 0.01$ vs. normal, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. control diabetic; B. Quantitative real time PCR analysis of Txnip in the kidney. $^{**}p < 0.01$ vs. normal, $^{\#}p < 0.05$ vs. control diabetic.

in the TGP treatment groups were strikingly increased compared with those of the control diabetic group (**Figure 2B**).

Effects of TGP on ASK1 expression in the kidney of diabetic rats induced by STZ

ASK1 was observed in the glomerulus and tubulointerstitium. In the kidneys of normal rats, there was minimal staining for ASK1. In contrast, abundant ASK1 was expressed in the kidneys of the diabetic rats. This overexpression was attenuated in diabetic rats treated with TGP. These results indicated that treatment of diabetic rats with TGP significantly reduced the expression of ASK1. (**Figure 3A** and **Table 3**). **Figure 3B** shows the effect of TGP on renal ASK1 protein expression in diabetic rats induced by STZ. Densitometric analysis of the Western blot showed that the protein level of ASK1 was strikingly higher in the control diabetic rats than those in the normal rats. Compared with those in the control diabetic group, the expression of ASK1 was significantly decreased by TGP administration with 50, 100 and 200 mg/kg.

Effects of TGP on p-p38 MAPK expression in the kidney of diabetic rats induced by STZ

Figure 4 shows renal p-p38 MAPK protein markedly increased in control diabetic rats when compared with the normal rats, while the treatment with TGP with 50, 100 and 200 mg/kg markedly decreased its expression.

Discussion

As previously reported, the hallmarks of diabetic nephropathy (DN), such as the relative kidney weight, glomerular volume, urinary albumin excretion rate and tubulointerstitial damage indices all increased after 8 weeks post induction with STZ; while TGP

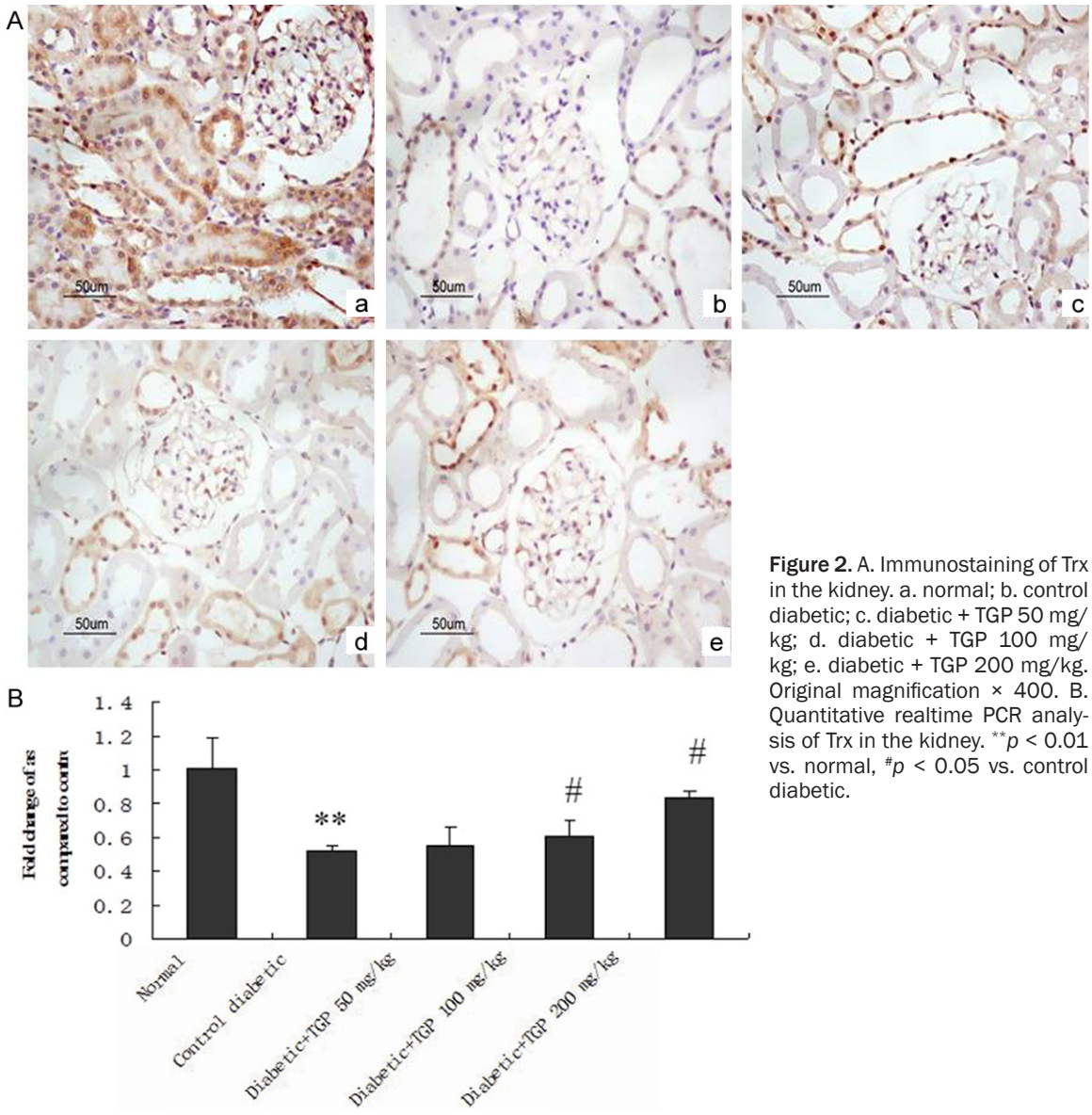

Figure 2. A. Immunostaining of Trx in the kidney. a. normal; b. control diabetic; c. diabetic + TGP 50 mg/kg; d. diabetic + TGP 100 mg/kg; e. diabetic + TGP 200 mg/kg. Original magnification × 400. B. Quantitative realtime PCR analysis of Trx in the kidney. $^{**}p < 0.01$ vs. normal, $^{\#}p < 0.05$ vs. control diabetic.

Table 2. Semiquantitative assessment of Trx immunohistochemisty staining in five groups of rats

Group	Dose (mg/kg)	Glomeruli (score)[a]	Tubulointerstitum (%)
Normal	-	2 (1~2)	25.31 ± 4.01
Control diabetic	-	0 (0~1)**	11.81 ± 3.64**
Diabetic + TGP	50	1 (0~2)	15.65 ± 4.08$^{\#}$
	100	1 (1~2)$^{\#\#}$	19.16 ± 2.15$^{\#\#}$
	200	1 (1~2)$^{\#\#}$	20.17 ± 3.91$^{\#\#}$

Data are expressed as means ± SEM. Number of rats in each group was 10. $^{**}p < 0.01$ compared with normal group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with control diabetic group. [a]Shown as the median. Statistical analysis was performed by ANOVA for normal distribution.

decreased albuminuria, alleviated glomerular and tubulointerstitial injuries, which suggested that TGP might have renoprotection effects without changing blood glucose and blood lipids levels [14, 15]. Previous researches have indicated that the protective effects of TGP in diabetic rats was related to anti-inflammatory and antioxidative role. In our present study, we further clarified effects of TGP on Trx system in STZ-induced diabetic rats. The aim of our study was to investigate the renoprotective mechanism of TGP from a different angle.

Oxidative stress, caused by a relative overload of oxidants and depletion of antioxidants, is implicated in the pathogenesis of DN [16]. In parallel, the Trx system plays an important role in the oxidative stress

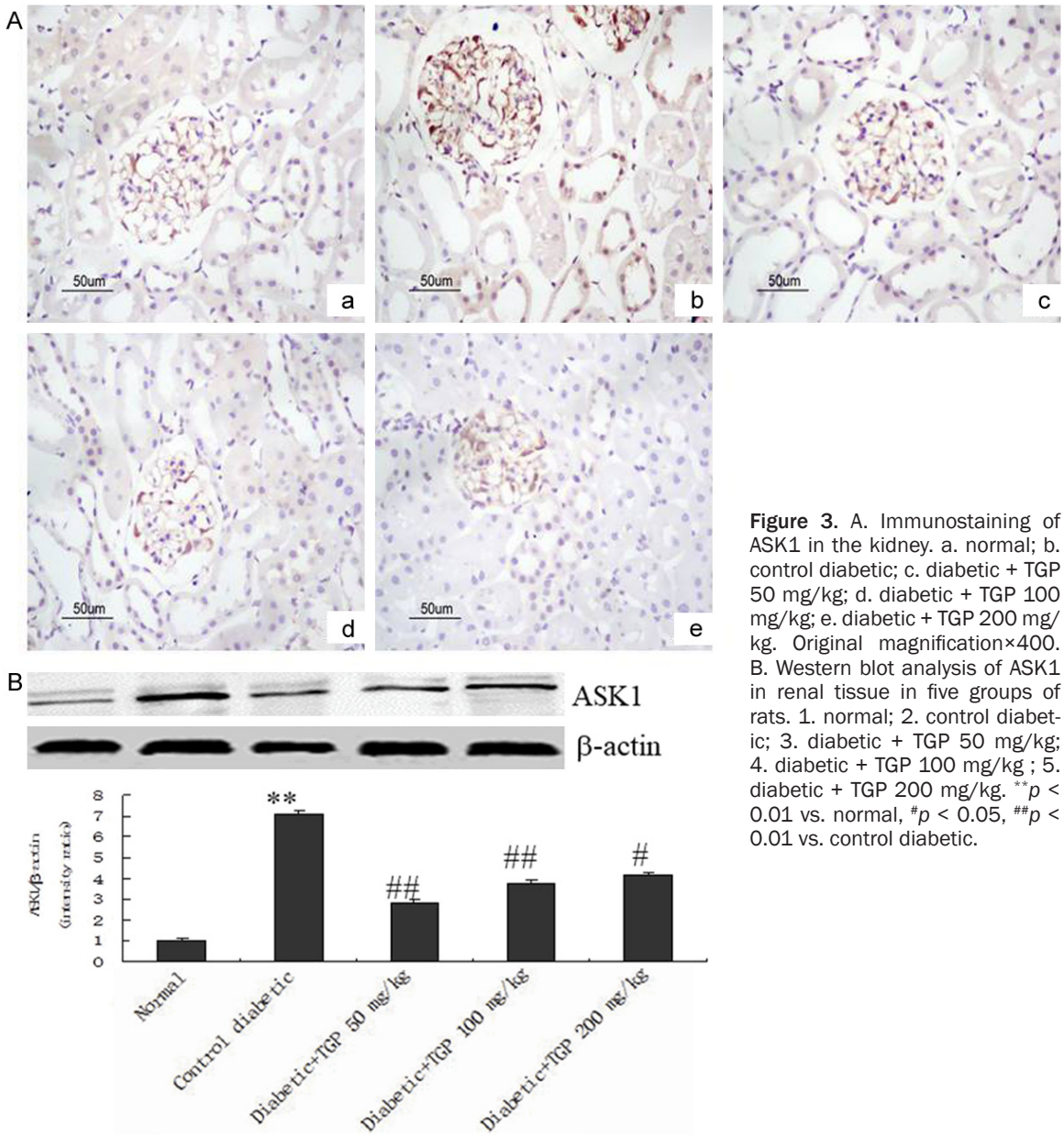

Figure 3. A. Immunostaining of ASK1 in the kidney. a. normal; b. control diabetic; c. diabetic + TGP 50 mg/kg; d. diabetic + TGP 100 mg/kg; e. diabetic + TGP 200 mg/kg. Original magnification×400. B. Western blot analysis of ASK1 in renal tissue in five groups of rats. 1. normal; 2. control diabetic; 3. diabetic + TGP 50 mg/kg; 4. diabetic + TGP 100 mg/kg ; 5. diabetic + TGP 200 mg/kg. $^{**}p < 0.01$ vs. normal, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. control diabetic.

Table 3. Semiquantitative assessment of ASK1 immunohistochemisty staining in five groups of rats

Group	Dose (mg/kg)	Glomeruli (score)[a]	Tubulointerstitum (%)
Normal	-	0.5 (0~1)	2.54 ± 0.63
Control diabetic	-	2.5 (1~3)**	8.48 ± 1.91**
Diabetic + TGP	50	2 (0~2)$^{\#}$	6.79 ± 1.43$^{\#}$
	100	1 (1~2)$^{\#\#}$	6.44 ± 1.26$^{\#\#}$
	200	1 (0~2)$^{\#\#}$	4.06 ± 0.92$^{\#\#}$

Data are expressed as means ± SEM. Number of rats in each group was 10. $^{**}p < 0.01$ compared with normal group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with control diabetic group. [a]Shown as the median. Statistical analysis was performed by ANOVA for normal distribution.

mechanism [17]. Trx, an important member of the Trx system, alleviates oxidative stress by scavenging ROS, and regulates a variety of redox-sensitive signaling pathways, such as c-Jun N-terminal kinase (JNK) and p38 MAPK pathways [18]. Txnip, the endogenous inhibitor of cellular Trx (Trx), exerts a critically important effects in “amplifying” inflammatory activation and apoptotic pathways. Ishrat et al. discovered that the Trx/Txnip system is involved in multiple signaling pathways, which are involved in a variety of cellular

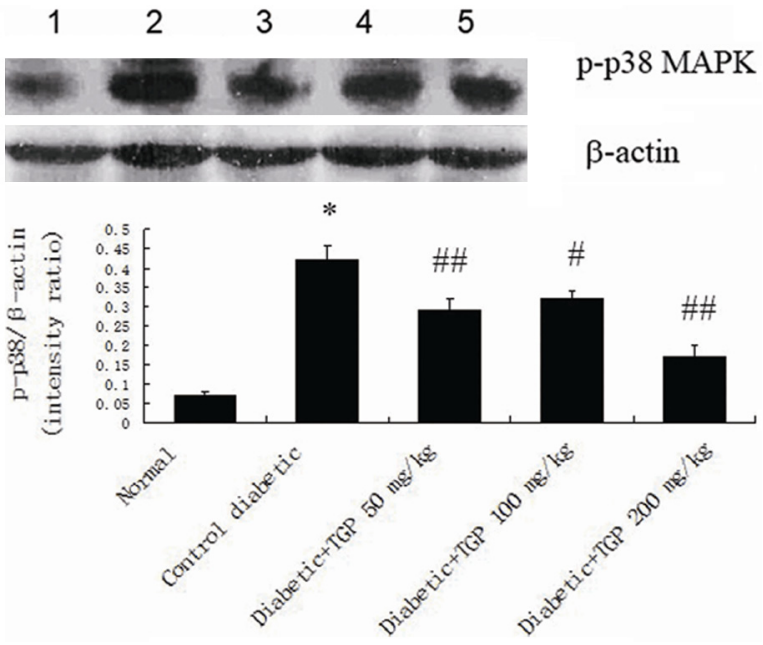

Figure 4. Western blot analysis of p-p38 MAPK in renal issue in five groups of rats. 1. normal; 2. control diabetic; 3. diabetic + TGP 50 mg/kg; 4. diabetic + TGP 100 mg/kg; 5. diabetic + TGP 200 mg/kg. $^{**}p < 0.01$ vs. normal, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. control diabetic.

functions. Txnip acts as a pro-apoptotic protein by interacting with ASK1, leading to activation of the p38 MAPK pathway and cell death [19]. Apoptosis signal-regulating kinase 1 (ASK1/MAP3K5), a member of the MAPKKK family which can induce activation of p38 and JNK, is markedly activated by ROS and plays a critical role in a variety of cellular responses induced by ROS, including cell apoptosis, growth, and differentiation [20]. Jung et al. indicated that Txnip binds Trx and acts as a competitive inhibitor, removing Trx from proteins those are negatively impacted by the steric effect of Trx-1 binding, such as ASK1 [21]. Saitoh et al. observed that Trx could combine with the N-terminal of ASK1 and form protein-protein complexes to inhibit the activity of ASK1 [22]. In summary, ASK1 released from Trx-ASK1 complexes due to the competive combination with Trx between Txnip and ASK1, then the dissociative ASK1 activates JNK and p38 MAPK from downstream and induces cell apoptosis [23]. On the other hand, p38 MAPK can upregulate the expression of Txnip and increase oxidative stress to promote the progress in DN [24]. Therefore, the inhibition of the p38 MAPK pathway could decrease the expression of Txnip, ASK1 and increase the expression of Trx and then slow down the progress of DN.

As an active compound extracted from the roots of Paeonia lactiflora Pall, TGP is identified to ameliorate clinical inflammatory reaction and has been widely used in the treatment of rheumatoid arthritis (RA) with a long history in traditional Chinese medicine [25]. Recently, TGP has been used to treat chronic nephritis, including Heymann nephritis and IgA nephropathy. Fang et al. observed that the induction of Txnip expression is dependent on the activation of p38 MAPK pathway [26]. In other words, the inhibition of the p38 MAPK pathway in hyperglycemic condition can substantially attenuate the expression of Txnip. p38 MAP kinase, a member of the family of serin/threonine kinases, is an important stress signaling molecule and is involved in the regulation of many cellular functions. Ren et al. demonstrated that p38 MAPK signaling may contribute to the initiation and progression of DN [27]. Moreover, our present research indicated that TGP can inhibit the activation of the p38 MAPK pathway in the kidneys of DN rats. In this study, the expression of Txnip and ASK1 was dramatically decreased in the diabetic model group treated with TGP in comparison with control diabetic group. Nevertheless, the expression of Trx was increased by TGP treatment. Therefore, we suppose that the anti-oxidation efficacy of TGP may possibly be involved in the regulation of the Trx system. TGP may exert the antioxidant effect by inhibiting the p38 MAPK pathway to decrease the expression of Txnip. Thus, the Trx levels were increased with the decreasing level of ASK1 and then the progression of DN was delayed.

Our findings show the significant role of TGP in the DN process and emphasizes the importance of antioxidative defense mechanisms in a complicated DN process. Moreover, the close contact between oxidative stress and the Trx system is confirmed. However, the specific mechanism that TGP acts on the Trx system has not been completely understood. Our future research will further clarify these questions.

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Disclosure of conflict of interest

None.

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