Original Paper

GSK-3beta Inhibitor Induces Expression of Nrf2/TrxR2 Signaling Pathway to Protect against Renal Ischemia/Reperfusion Injury in Diabetic Rats

Bo Hu^a Yuhong Wu^a Jie Liu^a Xiaohua Shen^a Fei Tong^b Guangtao Xu^b
Ruilin Shen^a

^aDepartment of Pathology and Nephrology, Jiaying Hospital of Traditional Chinese Medicine, Zhejiang Chinese Medical University; ^bDepartment of Pathology and Provincial Key Discipline of Pharmacology, Jiaying University Medical College, Jiaying, Zhejiang Province, P.R.China

Key Words

GSK-3beta inhibitor • Renal ischemia/reperfusion injury • Nrf2 • TrxR2 • Diabetes mellitus

Abstract

Background/Aims: Diabetes mellitus (DM) can lead to renal damage and dysfunction, and exacerbate renal ischemia/reperfusion injury (RI/RI). The aim of this study was to investigate the protective effect of GSK-3β inhibitor TDZD-8 against RI/RI through Nrf2/TrxR2 signaling pathway in a rat DM model. **Methods:** A DM rat model was established by a single injection of streptozocin. Diabetic rats were pretreated with TDZD-8 (1 mg/kg bw) or TDZD-8+auranofin (10 nmol/L, 5ml/kg bw), and then subjected to 45-min ischemia and 24-h reperfusion. Rats were equally randomized into four groups: a Sham-operated group, a RI/RI group, a TDZD-8 group, and a TDZD-8+auranofin group. Serum levels of BUN and Scr were measured. SOD activity, MDA content, and Nrf2, TrxR2 and caspase-3 expressions in rat kidney tissues were determined. **Results:** Renal function was improved, oxidative stress and cell apoptosis were reduced, and the expression of Nrf2 and TrxR2 was up-regulated in TDZD-8 treated rats as compared with those in auranofin treated rats. **Conclusion:** TDZD-8 may exert its protective effect against RI/RI by regulating the Nrf2/TrxR2 signaling pathway in the kidney tissue in DM.

© 2016 The Author(s)
Published by S. Karger AG, Basel

Bo Hu and Yuhong Wu contributed equally to this study and therefore shared first authorship.

Guangtao Xu and Ruilin Shen

Department of Pathology and Nephrology, Jiaying University Medical College, Jiaying Hospital of Traditional Chinese Medicine, 1501 East Zhongshan Road, Jiaying 314001, Zhejiang (P.R.China), E-Mail: tsuigt@126.com and shenrldm@sina.com

Introduction

Chronic kidney disease (CKD) is a major complication of diabetes mellitus (DM), and approximately one-third of *diabetic* patients [1, 2]. DM may lead to renal damage and dysfunction, and exacerbates renal ischemia/reperfusion (I/R) injury (RI/RI) [3]. Despite the improvements in renal protection over years, renal failure after RI/RI remains a challenging clinical problem.

Nuclear-factor-E2-related factor 2 (Nrf2) is a member of the basic leucine zipper domain (bZIP) transcription factors that is widely expressed in many tissues. Activation of antioxidant genes is mostly dependent upon Nrf2 [4, 5]. Under normal circumstances, Nrf2 combines with Kelch-like ECH-associated protein-1 (Keap-1, a thiol-rich protein) in the cytoplasm. The thiol of Keap-1 derives from a crosslink after receiving the extracellular signaling, leading Nrf2 into the nucleus, where Nrf2 participates in the transcription of the downstream II metabolic enzyme genes, including thioredoxin reductase-2 (TrxR2) [6].

Therefore, Nrf2 was reported to have a protective effect against inflammation and oxidation in cells via the production of TrxR2 [7-9]. TrxR is one of the important enzymes to be resistant to oxidative stress in mitochondria [10]. When cells or tissues suffer from oxidative stress stimulation, accumulation of reactive oxygen species (ROS) or activation of extracellular signal regulating kinase (ERK) may induce the release and transfer of Nrf2 to the nucleus, which may further trigger high expression of downstream coding phase II detoxification enzymes and drug flow pump gene by modulation of Nrf2, Trx and TrxR [6, 11, 12]. The important system of redox is made up of Trx, TrxR, and NADPH in cells, and TrxR can inhibit cell apoptosis through maintaining the reduced state of Trx [13-16].

GSK-3 β inhibitor, 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (TDZD-8) has been reported to reduce enteral and hepatic I/R injury through inhibiting apoptosis [17, 18]. However, it is not known whether TDZD-8 could also play a role in protecting the diabetic kidney and inducing the expressions of Nrf2/TrxR2 in diabetic rats to inhibit renal damage from I/R-induced injury. The aim of the present study was to explore the possible role of TDZD-8 in protecting against renal damage in a RI/RI model in diabetic rats, and investigate the potential effect of auranofin on I/R-induced renal injury.

Materials and Methods

Animal models

Forty Sprague-Dawley (SD) rats (180-220g) were obtained from the Animal Research Center of Jiaying University Medical College (Jiaying, China). A single dose of streptozocin (STZ, 50 mg/kg bw in citrate buffer, PH 4.5) was injected into the abdominal cavity of rats to generate the diabetes rat model. After 72 h, the serum level of glucose was confirmed using glucose oxidase-peroxidase (GOD-POD) methods [19]. The animals were monitored for 2 weeks, and rats with the glucose level from 16.7 mmol/L to 25.6 mmol/L were used in the following experiments. Subsequently, rats were equally randomized into 4 groups: (1) sham-operated group: the animals were treated with the bilateral renal arteries and veins were only separated and then treated with 10% dimethyl sulfoxide (DMSO, 1 ml/kg bw, i.v.). (2) RI/RI group: the animals were treated with ischemia through occluding the bilateral renal arteries and veins for 45 min and then 24 h-reperfusion was followed with DMSO (1 ml/kg bw, i.v.); (3) TDZD-8 group: animals were treated with 45 min-ischemia followed by 24 h-reperfusion with TDZD-8 (1 mg/kg bw, i.v., dissolved in DMSO) at 5 min prior to reperfusion; (4) TDZD-8+auranofin group: auranofin (5ml/kg bw, i.v., 10 nmol/L, dissolved in DMSO) was administered in the animals through the carotid artery to the renal artery at 1 h prior to the operation, and TDZD-8 was administered into animals based on the procedures of TDZD-8 group. Auranofin, TDZD-8, and STZ were purchased from Sigma-Aldrich (Cat. No. 18883-66-4, St Louis, USA). Animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of Jiaying University Medical College. The protocol was approved by the Animal Experiment Ethics Committee of Jiaying University Medical College.

Sample processing

The blood samples were collected via the abdominal aorta after reperfusion, and centrifuged at 3600× *g* for 15 min to harvest the sera. The whole kidneys were removed and immediately stored at -20 °C for analysis. All the left kidneys per group were used for histopathologic evaluation, and the right kidneys per group were homogenized in ice-cold normal saline (NS) and centrifuged at 3,600× *g* for 15 min to harvest the supernatant. The supernatant was stored at -80 °C for molecular biological detection.

Histopathological evaluation

The left kidneys per group were fixed in 4% paraformaldehyde overnight, rinsed by running water, dehydrated by a serial gradient of alcohol and paraffin embedded. The formalin-fixed paraffin-embedded tissues were sliced to 4-μm sections and hematoxylin and eosin (HE) stained. The samples were blindly analyzed by a pathologist. Briefly, 24 areas corresponding to the kidney proximal tubules were graded for the degree of renal damage based on the following parameters: tubular cell necrosis, cytoplasmic vacuole formation, hemorrhage, and tubular dilatation. Specifically, one whole deep coronal section was examined under the microscope and graded according to the extent of damage based on the percentage of involvement of the kidney. Higher scores represent more severe damage in a four-score system: 0, histopathological changes <10%; 1, = 10-25%; 2, =25-50%; 3, =50-75%; and 4, =75-100%. The mean score for each parameter was calculated and subjected to statistical analysis [20, 21].

Assessment of renal function

The levels of BUN and Scr were used to evaluate the renal function. BUN and Scr levels were measured using picric acid method according to the manufacturer's instructions [21]. Briefly, the blood samples were obtained after 24 h-reperfusion and centrifuged to harvest the sera and then measured with a Hitachi automatic biochemical analyzer 7060 c (Hitachi, Japan) employing a kit provided by Roche Diagnostics (Mannheim, Germany).

Antioxidative activity in the renal tissue

The antioxidative activity was detected with the SOD activity and MDA content in the kidney tissue homogenate, and measured at 550 and 532 nm following the xanthine oxidase and thiobarbituric acid method (Roche, Germany) [21, 22]. The level of lipid peroxides was expressed as U of SOD/mg protein and nmol of MDA/mg protein.

Measurement of TNF-α level

The level of TNF-α in the kidney tissue was measured to assess inflammatory injury as described previously [22]. TNF-α level was measured at 495 nm with a double antibody sandwich ELISA kit (Roche, Germany) according to the manufacturer's instructions. The level of TNF-α was expressed as ng of TNF-α/g protein.

Western blot

Western blot analysis was conducted following the reported methods [23-25]. Briefly, the renal tissue was homogenized in protein lysate buffer. The homogenate was resolved on 10% SDS-PAGE gels, and then electrophoretically transferred to the nitrocellulose membrane. After blocking with 3% BSA for 1h, the membranes were incubated with primary antibodies (against active Nrf2, TrxR2, and caspase-3) at 4 °C overnight, and subsequently with alkaline phosphatase-conjugated secondary antibodies. The membranes were developed by 5-b-romo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium. Blots were stained with anti-β-actin and anti-GAPDH antibodies, and the levels of proteins were normalized with respect to β-actin band and GAPDH band density. All protein rabbit IgG antibodies were provided by Roche Diagnostics (Mannheim, Germany). The antigen-antibody products were detected with Thermo Scientific Super Signal West Pico Chemiluminescent Substrate (Thermo Scientific, USA). The results were analyzed with a Fluor Chem system (Alpha Innotech, USA).

Fig. 1. Histopathologic evaluation of kidney injury. Light microscope images (HE, $\times 400$) *a*, Sham-operated group; *b*, RI/RI group; *c*, TDZD-8 group; and *d*, TDZD-8+auranofin group. Quantitative injury scores (QIS), expressed as mean $\pm$ SD, and are shown in Fig. 1e. * $P < 0.01$ vs. Sham-operated group; # $P < 0.01$, ## $P < 0.05$ vs. RI/RI group.

Statistical analysis

Statistical analysis was performed using the Microsoft Excel 2013 database and the SPSS 19.0 software. Data were expressed as the mean $\pm$ standard deviation (SD) and analyzed by the ANOVA with post-hoc testing and Pearson correlation analysis. A P value of less than 0.05 ($P < 0.05$) was regarded as statistically significant.

Results

Histopathological alterations at different treated groups

Tubular cell necrosis, cytoplasmic vacuoles, hemorrhage and tubular dilatation were observed in HE-stained sections in RI/RI group (Fig. 1b) but absent in Sham-operated group (Fig. 1a). Histological alterations were markedly reduced in the specimen of TDZD-8 group (Fig. 1c) as compared with RI/RI group. Histological alterations were moderately reduced in the specimen of TDZD-8+auranofin group as compared with RI/RI group (Fig. 1d). The corresponding quantitative analysis showed that the mean injury score in all drug treated animals was significantly higher than that in Sham-operated group; the mean injury score in RI/RI treated animals was significantly higher than that in TDZD-8 and TDZD-8+auranofin groups; the mean injury score in TDZD-8+auranofin group was significantly higher than that in TDZD-8 group (Fig. 1e).

Serum level of BUN and Scr

Serum levels of BUN and Scr in RI/RI group were significantly higher than those in Sham-operated group ($P < 0.01$) (Fig. 2). The mean BUN level was 6.06 ± 0.24 mmol/L in Sham-operated group vs. 35.18 ± 2.59 mmol/L in RI/RI group, which was reduced to 21.17 ± 3.18 mmol/L after administration of TDZD-8 ($P < 0.01$), and to 28.79 ± 2.52 mmol/L after administration of TDZD-8+auranofin ($P < 0.05$) (Fig. 2a). The mean Scr level in Sham-operated group was 28.34 ± 2.60 μ mol/L vs. 118.36 ± 3.70 μ mol/L in RI/RI group, which was reduced to 63.27 ± 3.61 μ mol/L after administration of TDZD-8 ($P < 0.01$), and to 85.65 ± 4.71 μ mol/L after administration of TDZD-8+auranofin ($P < 0.05$) (Fig. 2b).

Fig. 2. Serum level of BUN and Scr. Results are expressed as mean $\pm$ SD. * P <0.01 vs. Sham-operated group; # P <0.01, ## P <0.05 vs. RI/RI group.

Fig. 3. The activity of SOD and the level of MDA in renal tissues. Data are shown as mean $\pm$ SD. * P <0.01 vs. Sham-operated group; # P <0.01, ## P <0.05 vs. RI/RI group.

SOD activity and MDA content in the renal tissue

Renal tissue SOD activity in RI/RI group was significantly lower than that in Sham-operated group (12.23 ± 2.16 U/ mg protein vs. 41.24 ± 2.62 U/mg protein, P <0.01) (Fig. 3). Renal tissue SOD activity in TDZD-8 group (28.38 ± 2.57 U/mg protein) and TDZD-8+auranofin group (19.19 ± 1.61 U/mg protein) was significantly higher than that in RI/RI group (P <0.01 and P <0.05) (Fig. 3a). Renal tissue MDA content in RI/RI group was significantly higher than that in Sham-operated group (16.44 ± 3.14 nmol/mg protein vs. 4.12 ± 0.15 nmol/mg protein, P <0.01). It was significantly reduced in TDZD-8 group (9.23 ± 1.63 nmol/mg protein, P <0.01), and TDZD-8+auranofin group (12.28 ± 2.53 nmol/mg protein, P <0.05) as compared with that in RI/RI group (Fig. 3b).

TNF- α level in the renal tissue

TNF- α level in the renal tissue of RI/RI group was significantly higher than that in Sham-operated group (0.84 ± 0.004 ng/g protein vs. 0.48 ± 0.002 ng/g protein, $P < 0.01$). It was reduced significantly in TDZD-8 group (0.60 ± 0.014 ng/g protein, $P < 0.01$), and TDZD-8+auranofin group (0.70 ± 0.007 ng/g protein, $P < 0.05$) as compared with that in RI/RI group (Fig. 4).

The expression of Nrf2, TrxR2 and caspase-3 in the renal tissue

The expression of Nrf2 in the renal tissue of RI/RI group was significantly reduced as compared with that of Sham-operated group ($P < 0.01$). It was up-regulated significantly in TDZD-8 group as compared with that in RI/RI group ($P < 0.05$) (Fig. 5a). The expression of TrxR2 in the renal tissue of RI/RI group was significantly higher than that in Sham-operated group ($P < 0.05$). It was up-regulated significantly in TDZD-8 group and moderately in TDZD-8+auranofin group as compared with that in RI/RI group ($P < 0.01$ and $P < 0.05$) (Fig. 5b). The expression of caspase-3 in the renal tissue of RI/RI group was significantly higher than that in Sham-operated group ($P < 0.01$). It was reduced significantly in TDZD-8 group, and moderately in TDZD-8+auranofin group as compared with that in RI/RI group ($P < 0.01$ and $P < 0.05$) (Fig. 5c).

Correlation analysis

Correlation analysis among BUN, Scr, SOD, MDA, TNF- α content and Nrf2, TrxR2, and caspase-3 expression showed that serum BUN and Scr levels were negatively correlated with SOD activity ($r = -0.702$, $P < 0.01$), Nrf2 expression ($r = -0.687$, $P < 0.01$), and TrxR2 expression ($r = -0.674$, $P < 0.01$), and positively correlated with MDA content ($r = 0.823$, $P < 0.01$), TNF- α ($r = 0.799$, $P < 0.01$) and the level of caspase-3 expression ($r = 0.831$, $P < 0.01$).

Discussion

GSK-3 β is a serine/threonine protein kinase that participates in the regulation of cellular functions [26-28]. Upon external stimulation, GSK-3 β is activation and phosphorylated, which may reduce enzyme activities and change the activity or function of its downstream components. Recent studies suggested that the inhibition of GSK-3 β could reduce inflammation of endothelial cells in the brain, and the level of acute systemic proinflammatory cytokines [26-28]. Several studies reported that TDZD-8 could reduce I/R injury of the intestines and brain due to the inhibition of inflammation [18, 29]. Other studies demonstrated that TDZD-8 could reduce the level of TNF- α and restrain cell apoptosis [18, 30].

It was found in the present study that RI/RI caused renal tissue damage accompanied with elevation of serum BUN and Scr levels. Administration of TDZD-8 attenuated the tissue damage in RI/RI treated animals, whereas auranofin exacerbated the tissue damage in RI/RI group to some extent. It was reported in previous studies that RI/RI increased the production of ROS and dependent signaling cascades, reduced SOD activity, and increased the level of

Fig. 4. The level of TNF- α in renal tissues. Data are expressed as mean $\pm$ SD. * $P < 0.01$ vs. Sham-operated group; # $P < 0.01$, ## $P < 0.05$ vs. RI/RI group.

Fig. 5. The expression of Nrf2, TrxR2, and caspase-3 in renal tissues. Data were expressed as mean $\pm$ SD. (a) * P <0.01 vs. Sham-operated group; # P <0.05 vs. RI/RI group. (b) * P <0.05 vs. Sham-operated group; # P <0.01, ## P <0.05 vs. RI/RI group. (c) * P <0.01 vs. Sham-operated group; # P <0.01, ## P <0.05 vs. RI/RI group.

MDA [31-33]. It was found in the present study that TDZD-8 could improve SOD activity and decrease the level of MDA content, thus attenuating renal oxidative injury, and that auranofin could antagonize the effect of TDZD-8. Correlation analysis showed that the levels of serum BUN and Scr were negatively correlated with SOD activity and positively correlated with the level of MDA content. These results verify the above injury mechanism in the RI/RI model.

TrxR, a dimer enzyme of NADPH and a family member of disulfide flavoprotein redox enzyme, contains a FAD structural domain [34]. TrxR possesses a variety of anti-inflammatory and cytoprotective activities. The expression of TrxR is upregulated to protect cells against injury under oxidative stress [35]. Therefore, the induction of TrxR is considered to be an adaptive process when cells suffer from various forms of oxidative stress. Nrf2, a key regulator of the antioxidative defense pathway, can be activated in STZ-induced diabetes and diabetic neuropathy [36-39]. The induction of TrxR2 is an adaptive cellular defense response against oxidative stress and protection of cells in pathophysiological states [40]. The results of the present study showed that TDZD-8 supplementation partially recovered the expression of TrxR2, Nrf2, and cytoprotective anti-oxidant enzyme. TDZD-8 could ameliorate renal tissue damage by reducing the levels of BUN, Scr, MDA, and TNF- α , and at the same time increasing the activity of SOD, and upregulating expression of Nrf2 and TrxR2 against damage. Inhibition of TrxR2 by auranofin could downregulate the expression of TrxR2. These results indicate

that the expression of TrxR2 depends on the expression of Nrf2. TDZD-8 upregulated the expression of TrxR2 by activating Nrf2 in the kidney to exert its anti-inflammatory, antioxidant and cytoprotective effects. Several studies have demonstrated that TrxR2 could reduce the level of TNF- α and the expression of caspase-3, thus reducing oxidative stress [23-25, 41, 42]. Our data suggested that the protective mechanism of TDZD-8 lies in its anti-inflammatory activity by inhibiting the release of proinflammatory mediators, and its anti-oxidative activity by reducing the productions of lipid peroxidation and antiapoptotic activity, which probably depends on downregulation of activated caspase-3 and TNF- α via Nrf2/TrxR2 signaling pathway on RI/RI in DM.

Conclusion

In summary, our data showed that RI/RI in DM is accompanied with hyperglycemia, oxidative stress, decreased expression of Nrf2 and TrxR2, and increased expression of caspase-3 and TNF- α level. TDZD-8 could partially suppress the expression of caspase-3 and enhanced the expression of Nrf2 and TrxR2. TDZD-8 could reduce the severity of oxidative stress, inflammatory activity, and apoptotic activity through activating antioxidative defense signaling pathway on RI/RI in DM.

Disclosure Statement

The authors state that they have no conflict of interests and nothing to disclose.

Acknowledgements

The authors appreciate the support from the Science and Technology Planning Project of Zhejiang Province (2015C37130); the Zhejiang Provincial Natural Science Foundation (LQ14H230001); and the Science and Technology Planning Project of Jiaxing (2012AY1072-3).

References

- 1 Ritz E, Rychlik I, Locatelli F, Halimi S: End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. *Am J Kidney Dis* 1999;34:795-808.
- 2 Haider DG, Sauter T, Lindner G, Masghati S, Peric S, Friedl A, Wolzt M, Horl WH, Soleiman A, Exadaktylos A, Fuhrmann V: Use of calcium channel blockers is associated with mortality in patients with chronic kidney disease. *Kidney Blood Press Res* 2015;40:630-637.
- 3 Chodavarapu H, Grobe N, Somineni HK, Salem ES, Madhu M, Elased KM: Rosiglitazone treatment of type 2 diabetic db/db mice attenuates urinary albumin and angiotensin converting enzyme 2 excretion. *PLoS One* 2013;8:e62833.
- 4 Kang KW, Lee SJ, Kim SG: Molecular mechanism of nrf2 activation by oxidative stress. *Antioxid Redox Signal* 2005;7:1664-1673.
- 5 Zhang G, Wang Q, Zhou Q, Wang R, Xu M, Wang H, Wang L, Wilcox CS, Liu R, Lai EY: Protective effect of tempol on acute kidney injury through pi3k/akt/nrf2 signaling pathway. *Kidney Blood Press Res* 2016;41:129-138.
- 6 Copple IM, Goldring CE, Kitteringham NR, Park BK: The nrf2-keap1 defence pathway: Role in protection against drug-induced toxicity. *Toxicology* 2008;246:24-33.
- 7 Hashemy SI, Holmgren A: Regulation of the catalytic activity and structure of human thioredoxin 1 via oxidation and s-nitrosylation of cysteine residues. *J Biol Chem* 2008;283:21890-21898.

- 8 Meyer Y, Buchanan BB, Vignols F, Reichheld JP: Thioredoxins and glutaredoxins: Unifying elements in redox biology. *Annu Rev Genet* 2009;43:335-367.
- 9 Zhang G, Zou X, Huang Y, Wang F, Miao S, Liu G, Chen M, Zhu Y: Mesenchymal stromal cell-derived extracellular vesicles protect against acute kidney injury through anti-oxidation by enhancing nrf2/are activation in rats. *Kidney Blood Press Res* 2016;41:119-128.
- 10 Forman HJ, Fukuto JM, Miller T, Zhang H, Rinna A, Levy S: The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. *Arch Biochem Biophys* 2008;477:183-195.
- 11 Maher JM, Cheng X, Slitt AL, Dieter MZ, Klaassen CD: Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. *Drug Metab Dispos* 2005;33:956-962.
- 12 Hayashi A, Suzuki H, Itoh K, Yamamoto M, Sugiyama Y: Transcription factor nrf2 is required for the constitutive and inducible expression of multidrug resistance-associated protein 1 in mouse embryo fibroblasts. *Biochem Biophys Res Commun* 2003;310:824-829.
- 13 Fujino G, Noguchi T, Takeda K, Ichijo H: Thioredoxin and protein kinases in redox signaling. *Semin Cancer Biol* 2006;16:427-435.
- 14 Roberts PJ, Der CJ: Targeting the raf-mek-erk mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene* 2007;26:3291-3310.
- 15 Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H: Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ask) 1. *EMBO J* 1998;17:2596-2606.
- 16 Li X, Xie Z, Lin M, Huang R, Liang Z, Huang W, Jiang W: Renalase protects the cardiomyocytes of sprague-dawley rats against ischemia and reperfusion injury by reducing myocardial cell necrosis and apoptosis. *Kidney Blood Press Res* 2015;40:215-222.
- 17 Takada Y, Fang X, Jamaluddin MS, Boyd DD, Aggarwal BB: Genetic deletion of glycogen synthase kinase-3beta abrogates activation of ikappabalpha kinase, jnk, akt, and p44/p42 mapk but potentiates apoptosis induced by tumor necrosis factor. *J Biol Chem* 2004;279:39541-39554.
- 18 Cuzzocrea S, Mazzon E, Esposito E, Muia C, Abdelrahman M, Di Paola R, Crisafulli C, Bramanti P, Thiemeermann C: Glycogen synthase kinase-3beta inhibition attenuates the development of ischaemia/reperfusion injury of the gut. *Intensive Care Med* 2007;33:880-893.
- 19 Zhu D, Zhang L, Cheng L, Ren L, Tang J, Sun D: Pancreatic kininogenase ameliorates renal fibrosis in streptozotocin induced-diabetic nephropathy rat. *Kidney Blood Press Res* 2016;41:9-17.
- 20 He X, Xu X, Fan M, Chen X, Sun X, Luo G, Chen L, Mu Q, Feng Y, Mao Q, Chao Z: Preconditioning with hyperbaric oxygen induces tolerance against renal ischemia-reperfusion injury via increased expression of heme oxygenase-1. *J Surg Res* 2011;170:e271-277.
- 21 Tong F, Luo L, Liu DJ: Effect of intervention in mast cell function before reperfusion on renal ischemia-reperfusion injury in rats. *Kidney Blood Press Res* 2016;41:335-344.
- 22 He FF, Li HQ, Huang QX, Wang QY, Jiang HJ, Chen S, Su H, Zhang C, Wang YM: Tumor necrosis factor-alpha and 8-hydroxy-2'-deoxyguanosine are associated with elevated urinary angiotensin-2 level in type 2 diabetic patients with albuminuria. *Kidney Blood Press Res* 2015;40:355-365.
- 23 Lopert P, Day BJ, Patel M: Thioredoxin reductase deficiency potentiates oxidative stress, mitochondrial dysfunction and cell death in dopaminergic cells. *PLoS One* 2012;7
- 24 Campbell L, Howie F, Arthur JR, Nicol F, Beckett G: Selenium and sulforaphane modify the expression of selenoenzymes in the human endothelial cell line eahy926 and protect cells from oxidative damage. *Nutrition* 2007;23:138-144.
- 25 Maldonado PD, Perez-De La Cruz V, Torres-Ramos M, Silva-Islas C, Lecona-Vargas R, Lugo-Huitron R, Blanco-Ayala T, Ugalde-Muniz P, Vazquez-Cervantes GI, Fortoul TI, Ali SF, Santamaria A: Selenium-induced antioxidant protection recruits modulation of thioredoxin reductase during excitotoxic/pro-oxidant events in the rat striatum. *Neurochem Int* 2012;61:195-206.
- 26 Ramirez SH, Fan S, Zhang M, Papugani A, Reichenbach N, Dykstra H, Mercer AJ, Tuma RF, Persidsky Y: Inhibition of glycogen synthase kinase 3beta (gsk3beta) decreases inflammatory responses in brain endothelial cells. *Am J Pathol* 2010;176:881-892.

- 27 Voelkl J, Mia S, Meissner A, Ahmed MS, Feger M, Elvira B, Walker B, Alessi DR, Alesutan I, Lang F: Pkb/sgk-resistant gsk-3 signaling following unilateral ureteral obstruction. *Kidney Blood Press Res* 2013;38:156-164.
- 28 Cuzzocrea S, Crisafulli C, Mazzon E, Esposito E, Muia C, Abdelrahman M, Di Paola R, Thiernemann C: Inhibition of glycogen synthase kinase-3 beta attenuates the development of carrageenan-induced lung injury in mice. *Br J Pharmacol* 2006;149:687-702.
- 29 Collino M, Thiernemann C, Mastrocola R, Gallicchio M, Benetti E, Miglio G, Castiglia S, Danni O, Murch O, Dianzani C, Aragno M, Fantozzi R: Treatment with the glycogen synthase kinase-3 beta inhibitor, tdzd-8, affects transient cerebral ischemia/reperfusion injury in the rat hippocampus. *Shock* 2008;30:299-307.
- 30 Gui B, Hua F, Chen J, Xu Z, Sun H, Qian Y: Protective effects of pretreatment with oleanolic acid in rats in the acute phase of hepatic ischemia-reperfusion injury: Role of the pi3k/akt pathway. *Mediators Inflamm* 2014;2014:451826.
- 31 Corsini E, Galbiati V, Nikitovic D, Tsatsakis AM: Role of oxidative stress in chemical allergens induced skin cells activation. *Food Chem Toxicol* 2013;61:74-81.
- 32 Hsu SP, Chen CC, Chien CT: Pretreatment of sialic acid efficiently prevents lipopolysaccharide-induced acute renal failure and suppresses tlr4/gp91-mediated apoptotic signaling. *Kidney Blood Press Res* 2016;41:267-277.
- 33 Li YJ, Zhong DD, Lei L, Jia YL, Zhou H, Yang BX: Propofol prevents renal ischemia-reperfusion injury via inhibiting the oxidative stress pathways. *Cell Physiol Biochem* 2015;37:14-26.
- 34 Benhar M: Nitric oxide and the thioredoxin system: A complex interplay in redox regulation. *Biochim Biophys Acta* 2015;1850:2476-2484.
- 35 Kabuyama Y, Kitamura T, Yamaki J, Homma MK, Kikuchi S, Homma Y: Involvement of thioredoxin reductase 1 in the regulation of redox balance and viability of rheumatoid synovial cells. *Biochem Biophys Res Commun* 2008;367:491-496.
- 36 Agca CA, Tuzcu M, Hayirli A, Sahin K: Taurine ameliorates neuropathy via regulating nf-kappa b and nrf2/ho-1 signaling cascades in diabetic rats. *Food Chem Toxicol* 2014;71:116-121.
- 37 Motohashi H, Yamamoto M: Nrf2-keap1 defines a physiologically important stress response mechanism. *Trends Mol Med* 2004;10:549-557.
- 38 Negi G, Kumar A, Joshi RP, Sharma SS: Oxidative stress and nrf2 in the pathophysiology of diabetic neuropathy: Old perspective with a new angle. *Biochem Biophys Res Commun* 2011;408:1-5.
- 39 Negi G, Kumar A, Sharma SS: Melatonin modulates neuroinflammation and oxidative stress in experimental diabetic neuropathy: Effects on nf-kappa b and nrf2 cascades. *J Pineal Res* 2011;50:124-131.
- 40 Patenaude A, Ven Murthy MR, Mirault ME: Mitochondrial thioredoxin system - effects of trxr2 overexpression on redox balance, cell growth, and apoptosis. *J Biol Chem* 2004;279:27302-27314.
- 41 Benhar M, Forrester MT, Hess DT, Stamler JS: Regulated protein denitrosylation by cytosolic and mitochondrial thioredoxins. *Science* 2008;320:1050-1054.
- 42 Kim JR, Lee SM, Cho SH, Kim JH, Kim BH, Kwon J, Choi CY, Kim YD, Lee SR: Oxidation of thioredoxin reductase in hela cells stimulated with tumor necrosis factor-alpha. *FEBS Lett* 2004;567:189-196.