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Troxerutin protects the mouse kidney from D-galactose-caused injury through anti-inflammation and anti-oxidation

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ABSTRACT

This study was carried out to investigate the protective effect of troxerutin against D-galactose (D-gal)-induced renal injury in mice. Hematoxylin and eosin (H&E) stained sections of kidneys revealedD-gal could cause renal injury and troxerutin could significantly attenuate the injury. We further investigated the mechanisms involved in the protective effects of troxerutin on mouse kidney. The following antioxidant defense enzymes were measured: cytosolic Cu/Zn superoxide dismutase (SOD-1), catalase (CAT) and glutathione peroxidase (GPx). The content of the lipid peroxidation product malondialdehyde (MDA) was also analyzed. In D-gal-treated mice, antioxidant enzymes activities were significantly decreased and the level of MDA was significantly higher than those in the vehicle controls. Our results indicated that the protective effect of troxerutin against D-gal induced renal injury might be caused, at least in part, by increasing the activity of antioxidant enzymes with a reduction in lipid peroxidation product. Furthermore, we also examined the inflammatory signal mediators of nuclear factor-κB (NF-κB), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and prostanoid receptor subtype EP2 by Western blot. After treatment with D-gal, the NF-κB p65, iNOS, COX-2 and EP2 were markedly upregulated. Upon co-treatment with the troxerutin, however, the expressions of the NF-κB p65, iNOS, COX-2 and EP2 markedly reduced, compared to D-gal treatment alone. These results indicated that troxerutin has significantly inhibitory effects on the NF-κB-mediated inflammatory response. These findings suggest troxerutin could attenuate renal injury induced by D-gal probably through its antioxidant and anti-inflammation properties.

1. Introduction

Reactive oxygen species (ROS) which causes extensive damage to DNA, proteins and lipids have been implicated in the initiation of various inflammation processes [1]. Many anti-oxidant defense systems consisting of enzymatic [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] and non-enzymatic (ascorbic acid, glutathione and α-tocopherol) compounds can maintain the balance between ROS generation and protection from damage by ROS. However, these defensive systems under conditions of severe oxidative stress do not protect against the attack of ROS, such as nitric oxide (NO) formed from Ca^{2+}-independent NO synthase (iNOS), which cause inflammation [2].

Studies have suggested that the expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) which are enzymes involved in the generation of inflammatory mediators can be regulated by several transcription factors, one of them is nuclear factor-kappa B (NF-κB) [3]. NF-κB is a ubiquitously expressed transcriptional regulator that controls the expression of genes involved in inflammatory responses [4]. Prostaglandin E_2 (PGE_2) is one of the most important arachidonate metabolites synthesized by the action of cyclooxygenases. The lipid mediator is involved in a wide range of diseases, including inflammation, by exerting pleiotropic actions [5]. The activity of PGE_2 is mediated by four receptors, termed E prostanoid receptors (EP1–EP4) [6].

D-galactose (D-gal) is a normal reducing sugar in the body. At the normal level, it is usually converted into glucose by galactose-1-phosphate uridyltransferase and galactokinase. However, at high levels, it can cause the accumulation of ROS, or stimulate free radical production indirectly by the formation of advanced glycation end products (AGEs) in vivo, finally resulting in oxidative stress [7,8]. Mice injected with D-gal have been used as an animal model of oxidative damage [9]. Recently, we found in the mouse model, oxidative damage associated with inflammatory damage.

Troxerutin, known as Vitamin P4, is a flavonoid present in tea, coffee, cereal grains and a variety of fruits and vegetables. It has aroused considerable interesting due to their broad pharmacological activities [10]. Troxerutin has been commonly used in the treatment of Chronic Venous Insufficiency (CVI) disease. It improves capillary function, reduces capillary fragility and abnormal leakage, and it has anti-erythrocytic, anti-

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thrombotic, fibrinolytic, odema-protective and rheological activity [11]. Recently, we firstly found that troxerutin could attenuate oxidative damage and inflammatory damage induced by D-gal in mouse liver (data not shown). Kidney is an important urinary organ in our body, and maintaining proper kidney function is essential to keep us healthy. Research showed that renal injury could be induced by injection of D-gal into normal mouse [12]. However, to our knowledge, it has not yet been reported that troxerutin could attenuate the renal injury in D-gal-treated mice. Hence the aim of the present study was to evaluate whether troxerutin has protective effect against D-gal-induced renal injury and to explore the mechanism of its action. We hypothesize that troxerutin might protect the kidney from D-gal-induced damage by attenuating oxidative stress and suppressing inflammation. Results in this study support our hypothesis, provide novel insights into the mechanisms of troxerutin in the protection of the kidney.

2. Materials and methods

2.1. Animals

Ten-week-old male Kunming strain mice (29.63±4.88 g) were purchased from the Branch of National Breeder Center of Rodents (Shanghai, China). Prior to experiments mice had free access to food and water and were kept under constant conditions of temperature (23±1 °C) and humidity (60%). Ten mice were housed per cage on a 12-hour light/dark schedule (lights on 08:30–20:30). After acclimatization to the laboratory conditions, mice were divided randomly into three groups, termed as group A, group B and group C. Mice in group B and group C received daily subcutaneous injections of D-gal (Sigma-Aldrich, MO, USA) at dose of 50 mg/kg/day for 8 weeks, and mice in group A served as vehicle control with injection of saline (0.9% NaCl) only. Then mice in group C received troxerutin (98.76%, Baoji Fangsheng Co., China) of 150 mg/kg/day in distilled water containing 0.1% Tween-80 (dH_2O/0.1% Tween-80) by oral gavage for another 4 weeks. Meanwhile, mice in group A and group B were given dH_2O/0.1% Tween-80 without troxerutin. All experiments were performed in compliance with the Chinese legislation on the use and care of laboratory animals and were approved by the respective university committees for animal experiments.

2.2. Histological analysis

The mice were deeply anesthetized with sodium pentobarbital and perfused transcardially with 25 ml of normal saline (0.9%). The kidney tissues were fixed in a fresh solution of 4% paraformaldehyde (pH 7.4) at 4 °C for 4 h, incubated overnight at 4 °C in 100 mM sodium phosphate buffer (pH 7.4) containing 30% sucrose, and embedded in Optimal Cutting Temperature (OCT, Leica, CA, Germany). Cryosections (12 μm) were collected on 3-aminopropyl-trimethoxysilane-coated slides (Sigma-Aldrich). Sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy (Axioskop 40, Zeiss, Germany) by a board-certified veterinary pathologist who was blind to the animals assigned to each experimental group.

2.3. Tissue homogenates

For biochemical studies, animals were deeply anesthetized and sacrificed. Kidneys were promptly dissected and perfused with 50 mM (pH 7.4) ice-cold phosphate buffered saline solution (PBS). Kidneys were homogenized in 1/5 (w/v) PBS containing a protease inhibitor cocktail (Sigma-Aldrich, MO, USA) with 10 strokes at 1200 rev/min in a Potter homogenizer. Homogenates were divided into two portions and one part was directly centrifuged at 8000 ×g for 10 min to obtain the supernatant. Supernatant aliquots were used to determine kidney CAT, GPx activities, MDA levels and protein contents. The second part of homogenates was sonicated four times for 30 s with 20 s intervals using a VWR Bronson Scientific sonicator, centrifuged at 5000 ×g for 10 min at 4 °C, and the supernatant was collected and stored at −70 °C for determination of Cu/Zn SOD enzyme activities. Protein contents were determined by using the BCA assay kit (Pierce Biotechnology Inc., Rockford, IL).

2.4. Measurement of malondialdehyde (MDA) level

Chemicals, including *n*-Butanol, thiobarbutiric acid, 1,1,3,3-tetramethoxy-propane and all other reagents, were purchased from Sigma Chemical Company. The level of MDA in kidney tissue homogenates was determined using the method of Uchiyama and Mihara [13]. Half a milliliter of homogenate was mixed with 3 ml of H_3PO_4 solution (1%, v/v) followed by addition of 1 ml of thiobarbituric acid solution (0.67%, w/v). Then the mixture was heated in a water bath at 95 °C for 45 min. The colored complex was extracted into *n*-butanol, and the absorption at 532 nm was measured using tetramethoxypropane as standard. MDA levels were expressed as nmol per mg of protein.

2.5. Measurement of Cu/Zn SOD activity

Chemicals used in the assay, including xanthine, xanthine oxidase, cytochrome *c*, bovine serum albumine (BSA) and SOD, were purchased from Sigma Chemical Company. SOD activity was measured according to the method described by Lu et al. [14]. Solution A was prepared by mixing 100 ml of 50 mM PBS (pH 7.4) containing 0.1 mM EDTA and 2 μmol of cytochrome *c* with 10 ml of 0.001 N NaOH solution containing 5 μmol of xanthine. Solution B contained 0.2 U xanthine oxidase/ml and 0.1 mM EDTA. Fifty microliters of a tissue supernatant was mixed with 2.9 ml of solution A and the reaction was started by adding 50 μl of solution B. Change in absorbance at 550 nm was monitored. A blank was run by substituting 50 μl of ultra pure water for the supernatant. SOD levels were expressed as U/mg protein with reference to the activity of a standard curve of bovine Cu/Zn SOD under the same conditions.

2.6. Measurement of CAT activity

CAT activity was assayed by the method of Aebi [15]. In brief, to a quartz cuvette, 0.65 ml of the phosphate buffer (50 mmol/l; pH 7.0) and 50 μl sample were added, and the reaction was started by addition of 0.3 ml of 30 mM hydrogen peroxide (H_2O_2). The decomposition of H_2O_2 was monitored at 240 nm at 25 °C CAT activity was calculated as nM H_2O_2 consumed/min/mg of tissue protein.

2.7. Measurement of GPx activity

The GPx activity assay was based on the method of Paglia and Valentine [16]. Tert-butylhydroperoxide was used as substrate. The assay measures the enzymatic reduction of H_2O_2 by GPx through consumption of reduced glutathione (GSH) that is restored from oxidized glutathione GSSG in a coupled enzymatic reaction by GR. GR reduced GSSG to GSH using NADPH as a reducing agent. The decrease in absorbance at 340 nm due to NADPH consumption was measured in a Molecular Devices M2 plate reader (Molecular Devices, Menlo. Park, CA). GPx activity was computed using the molar extinction coefficient of 6.22 mM^{-1} cm^{-1}. One unit of GPx was defined as the amount of enzyme that catalyzed the oxidation of 1.0 μmol of NADPH to $NADP^+$ per minute at 25 °C.

2.8. Protein and Western blot analysis

Total protein extracts were prepared in 3 ml of ice cold RIPA lysis buffer [1×TBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide] combining 30 μl of 10 mg/ml PMSF solution, 30 μl of Na_3VO_4 and 30 μl of protease inhibitors cocktail per gram of tissue.Lysates were

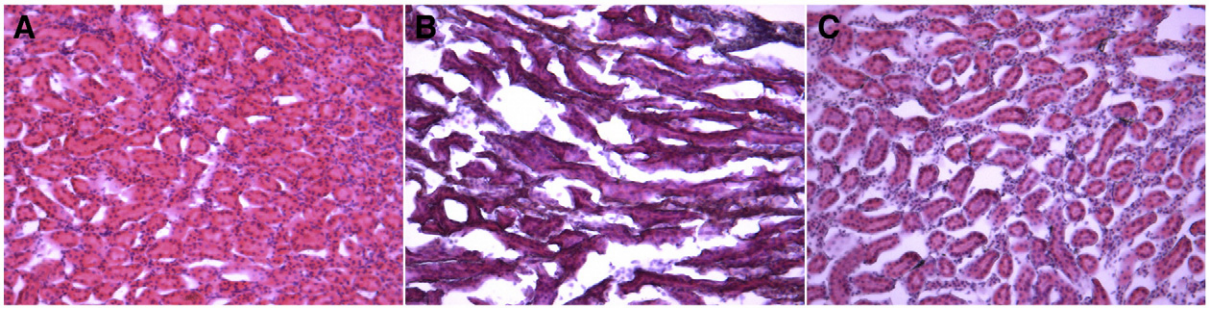

Fig. 1. Hematoxylin and eosin-stained kidneys from the treated mice. (A) The vehicle control mice; (B) D-gal-treated mice; (C) D-gal-treated mice fed with troxerutin. Original magnification, 100×.

centrifuged at 10,000 ×*g* for 10 min at 4 °C, and then remove the supernatants and centrifuge again. The supernatants were collected. Protein levels in the supernatants were determined using the BCA assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Samples (80 μg each) were separated by denaturing SDS–PAGE and transferred to a PVDF membrane (Roche Diagnostics Corporation, Indianapolis, IN, USA) by electrophoretic transfer. The membrane was pre-blocked with 5% non-fat milk and 0.1% Tween-20 in Tris-buffered saline (TBST), incubated overnight with the primary antibody (in TBST with 5% non-fat dried milk). Each membrane was washed three times for 15 min and incubated with the secondary horseradish perocidase-linked antibodies (Santa Cruz Biotechnology, CA and Cell Signaling Technology, Beverly, MA, respectively). Quantitation of detected bands was performed with the Scion Image analysis software (Scion Corp., Frederick, MD, USA). To prove equal loading, the blots were analysed for β-actin expression using an anti-β-actin antibody (Chemicon International Inc., Temecula, CA). Each density was normalized using each corresponding β-actin density as an internal control and averaged from the samples, and we standardized the density of vehicle control for relative comparison as 1.0 to compare other groups.

2.9. Statistical analysis

All statistical analyses were performed using the SPSS software, version 11.5. Analysis of variance (ANOVA) was carried out with Newman–Keuls or Tukey's HSD post hoc test for multiple comparisons. Data were expressed as means ± S.E.M. Statistical significance was set at $P<0.05$.

3. Results

3.1. Histological studies on effects of troxerutin in the D-gal-treated mice kidneys

The morphological features of haematoxylin and eosin (H&E) stained kidney sections are shown in Fig. 1. Severe structural damages were observed in the kidney structure of D-gal-treated mice as compared to that in the kidney of vehicle control mice. In D-gal-treated mice the renal sections showed extensive tubular damage by presence of necrotic epithelial cells. Significant number of nephrons with distended tubular lumen containing copious amounts of proteinaceous glomerular filtrate and characterized by pyknosis and karyolysis of tubular epithelial nuclei were observed. In some areas exfoliation of epithelial cells was observed in the tubular lumen. Intriguingly, troxerutin treatment could attenuate renal injury induced by D-galactose in mouse. The kidney showed no remarkable structural changes in D-gal-treated mice fed with troxerutin.

3.2. Effects of troxerutin on lipid peroxidation in D-gal-treated mice kidneys

Our results in Fig. 2 demonstrated that the level of MDA in the kidney of D-gal-treated mice was significantly higher than that in the vehicle control mice ($P<0.01$). The increase in lipid peroxidation indicates an elevated in vivo oxidative stress in the kidney of D-gal-treated mice. Interestingly, troxerutin could attenuate D-gal induced MDA increasing ($P<0.05$).

3.3. Effects of troxerutin on antioxidative status of D-gal-treated mice kidneys

Antioxidant enzymes, SOD, CAT and GPx, are thought to be effective for augmentation of antioxidant defenses. SOD can convert superoxide radicals to hydrogen peroxide and subsequently convert to water by CAT and GPx [17]. To determine whether the increased oxidative damages in the kidney of D-gal-treated mice are related to an altered antioxidant capacity, we measured the activities of major antioxidant enzymes in mouse kidney. Fig. 3 presents activities of antioxidant enzymes, including Cu/Zn SOD, CAT, GPx, in the kidneys of mice. In D-gal-treated mice, Cu/Zn SOD ($P<0.01$), CAT ($P<0.01$) and GPx ($P<0.001$) activities decreased significantly as compared with those in the vehicle controls. In contrast, no significant decrease in activities of these antioxidant enzymes was observed in D-gal-treated mice fed with troxerutin.

3.4. Effects of troxerutin on the expression of NF-κB p65, COX-2, iNOS and EP2 in the kidneys of D-gal-treated mice

To investigate the protective mechanism of troxerutin on the D-gal-treated mice, we examined the levels of NF-κB p65 by Western blotting. After treatment with D-gal, the DNA-binding activity of NF-κB p65 in nuclear extract fractions increased, compared to control (Fig. 4A, lane 2). However, this binding activity decreased upon the presence of troxerutin, compared to D-gal alone (Fig. 4A, lane 3).

Because NF-κB activation closely relates to its transcriptional activity on pro-inflammatory molecules, such as COX-2 and iNOS, we examined the inhibitory effects of troxerutin on D-gal-induced COX-2

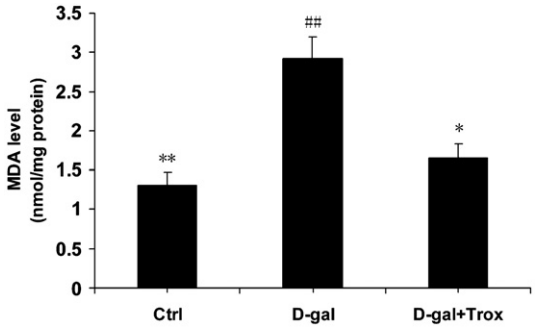

Fig. 2. The effects of troxerutin on the changes of MDA level induced by D-gal in the kidneys of mice. Each value is the means ± S.E.M. ($n=3$). *$P<0.05$; **$P<0.01$, as compared to the D-gal model; ##$P<0.01$, as compared to the control group.

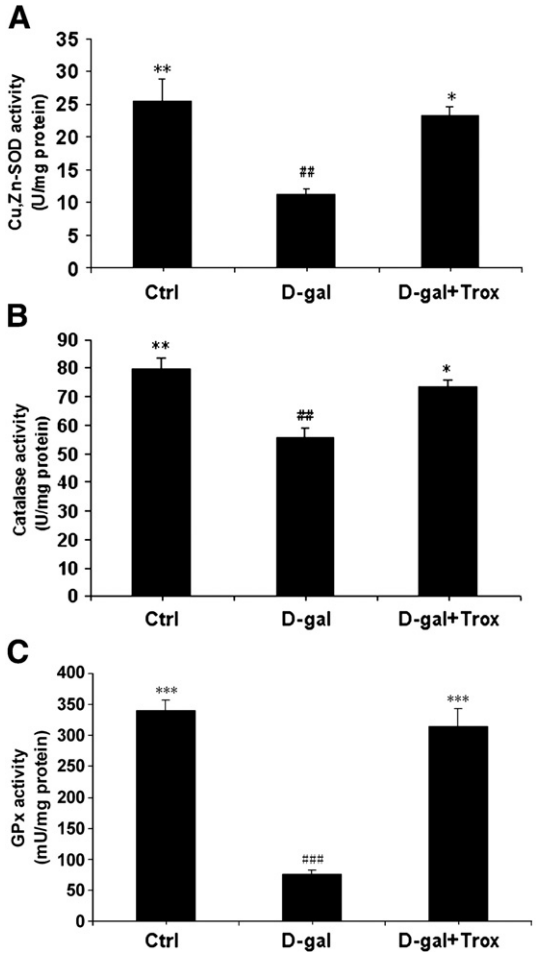

Fig. 3. The effects of troxerutin on the activity changes of Cu/Zn SOD (A), CAT (B), GPx (C) induced by D-gal in the kidneys of mice. Each value is the means ± S.E.M. ($n=3$). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, as compared to the D-gal model; $^{\#\#}P<0.01$; $^{\#\#\#}P<0.001$, as compared to the control group.

and iNOS expression in the kidneys using Western blot. After treatment with D-gal, the expressions of COX-2 and iNOS were markedly induced (Fig. 4A, lane 2). While as shown in Fig. 4, troxerutin was shown to markedly inhibit the expressions of these molecules in the kidneys of D-gal-treated mice. Upon co-treatment with the troxerutin, however, the intensities of the COX-2 and iNOS bands markedly reduced, compared to D-gal treatment alone (Fig. 4A, lane 3).

PGE_2, member of the autacoid family of lipid mediators, is a major renal cyclooxygenase product of arachidonic acid metabolism. PGE_2 binds to four G protein-coupled E-prostanoid receptors. The EP2 receptor belongs to the G-coupled prostanoid receptor superfamily. Western blot analysis showed the expression of EP2 receptor was induced by D-gal (Fig. 4A, lane 2) and was reversed by troxerutin (Fig. 4A, lane 3).

4. Discussion

The kidney is a highly specialized organ that maintains the internal environment of the body by selectively excreting or retaining various substances according to specific body needs. Lipopolysaccharide [18], alcohol [19], methamphetamine [20], glycerol [21], and many other drugs can induce renal injury, including oxidative stress and inflammation in mice and rats. D-gal-treated mice have been often used in antioxidative pharmacology research. Our laboratory has shown oxidative damage occurred in the brains of D-gal-treated mice [9,14]. Although the exact mechanism underlying D-gal-induced injury has not been defined, existing data indicate that the oxidative stress might be one of the reasons [22]. D-gal can cause the accumulation of reactive oxygen species (ROS), or stimulate free radical production indirectly by the formation of advanced glycation end products (AGEs) in vivo, finally resulting in oxidative damage [7]. It is reported that ROS can damage important biomolecules such as lipids, proteins, DNA and carbohydrates [23]. Lipid and protein peroxidation reactions play an important role in the pathogenesis of a variety diseases. Also, oxidative stress is caused by an imbalance of oxidants and antioxidants in favor of the former, and is capable of inflicting injury on membrane lipids, proteins and nucleic acids [24,25]. For the alleviation of multiple organ injury, evaluation and control of oxidative stress in vivo may become essential. A wide variety of functional assays are used in the field of research related with oxidative stress. However, direct detection of ROS and other free radicals is difficult, because these molecules are short-lived and highly reactive in a nonspecific manner [26]. Although ongoing oxidative damage is, thus, generally analyzed by measurement of secondary products, including derivatives of amino acids, nucleic acids, and lipid peroxidation, biomarkers to reflect minor changes in the pro-oxidant/antioxidant status under normal, nonpathological conditions might be of special interest. Antioxidant enzymes, SOD, CAT and GPx, are thought to be effective for augmentation of antioxidant defenses. SOD can convert superoxide radicals to hydrogen peroxide and subsequently convert to water by CAT and GPx [17]. Fig. 3 showed that D-gal-treated mice markedly decreased antioxidant ability of SOD, CAT and GPx activities, compared to the vehicle control. This result is consistent with that in our previous experiments [9,14]. MDA is a good indicator of the degree of lipid peroxidation [27]. Our results in Fig. 2 demonstrated that the level of MDA in the kidneys of D-gal-treated mice was significantly higher than that in the vehicle control mice. The above results indicated that D-gal could induce oxidative damage in the mouse kidney.

Inflammation is one of the leading causes of the many pathological states associated with oxidative stress [28]. Inflammation is a complex response of the immune system to a pathogen [29,30]. Although it plays an important role in the immune defense, it also contributes to the pathogenesis mediated by oxidative stress [31–33]. Immune injury to the kidney is well documented [31]. In the current study, we furthermore investigated the core molecules involved in the pathway of inflammation by western blots. COXs are rate-limiting enzymes in the metabolic pathway that converts arachidonic acid to prostaglandins, which are potent mediators of inflammation. Three COX isozymes were identified, and termed COX-1, COX-2 and COX-3. COX-2 is the isoform which is inducible at injury/inflammation sites and expressed constitutively in a few organs, such as the kidney [34]. COX-2 plays a role in the pathophysiological processes including inflammation [35,36]. Our results in Fig. 4B demonstrated that the level of COX-2 in the kidney of D-gal-treated mouse was significantly higher than that in the vehicle control mouse. It is supposed that, in various inflammatory events, nitric oxide (NO) may have a decisive function with cytokine activation under soft-tissue and bone damage conditions [37]. Although NO played an important role in the host defense against various pathogens, the overproduction of NO can be harmful and result in septic shock, rheumatoid arthritis, and autoimmune diseases [38]. Therefore, therapeutic agents that inhibit the iNOS may be useful for the relieving these inflammatory conditions. Fig. 4C showed that the level of iNOS in the kidney of D-gal-treated mouse was significantly higher than that in the vehicle control mouse. Expression of iNOS and COX-2 is largely regulated by transcriptional activation. Among these transcription factors, NF-κB, which is a primary transcription factor and regulates various genes, is important in the inflammation [39,40]. NF-κB is a redox-sensitive transcription factor that regulates a multitude of inflammatory genes, including cytokines, chemokines, adhesion molecules, and acute phase proteins. Under basal conditions, NF-κB is inactive and prevented from DNA binding and nuclear translocation by tight association in the cytoplasm with inhibitory proteins. Cell activation by a variety of extracellular signals such as oxidative stress, induces a cascade of events

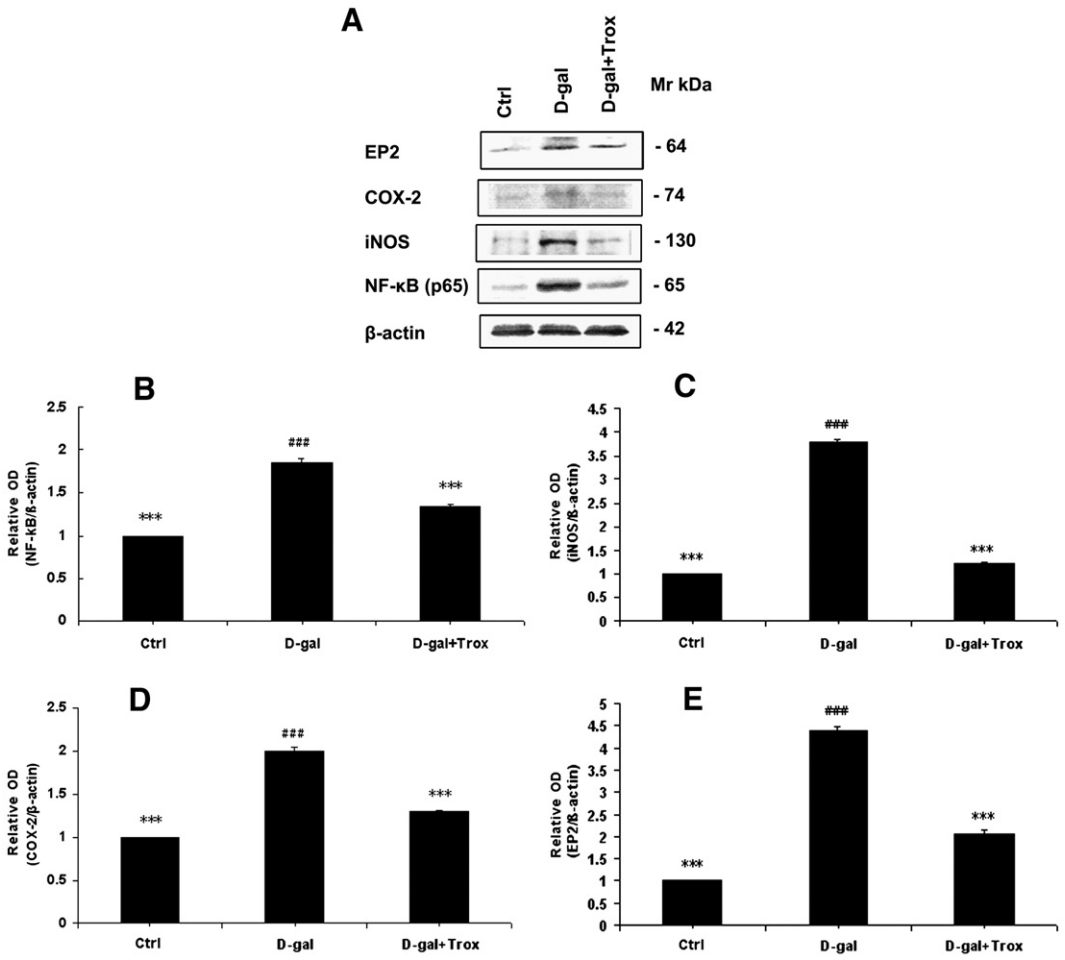

Fig. 4. Western blot analysis of the NF-κB p65, iNOS, COX-2 and EP2. (A) the effect of troxerutin on the expression of NF-κB p65, iNOS, COX-2 and EP2 in the kidneys of D-gal-treated mice. (B) relative density analysis of the NF-κB p65 bands. (C) relative density analysis of the iNOS protein bands. (D) relative density of the COX-2 protein bands. (E) relative density of the EP2 bands. β-Actin was probed as an internal control. The relative density is expressed as NF-κB/β-actin, iNOS/β-actin, COX-2/β-actin or EP2/β-actin ratio and the vehicle control is set as 1.0. Values are averages from three independent experiments. Each value is the means ± S.E.M. $^{***}P<0.001$, as compared to the D-gal model; $^{\#\#\#}P<0.001$, as compared to the control group.

that lead to activate NF-κB then translocates to the nucleus where it binds to DNA elements in the promoters of a number of proinflammatory gene families [41,42]. In our study, after treatment with D-gal, the expression of NF-κB p65 in nuclear extract fractions increased, compared to control (Fig. 4D). PGE_2 is an eicosanoid produced by COX that is usually considered as a potent inflammatory mediator [43]. PGE_2 and its signaling pathway has been the target of clinical utility for a variety of disease/pathophysiological conditions, such as kidney failure. However, systemic side effects have limited their clinical utility. The pharmacological activities of PGE_2 are mediated through the activation of G-protein coupled receptors, known as the EP receptors. There are four subtypes of EP receptors designated as EP1, EP2, EP3, and EP4. Some studies showed that induction of EPs is associated with the expression of proinflammatory enzymes such as iNOS and COX-2 [44]. But until now, the precise roles of each receptor were largely unknown. Development and testing of specific PGE_2 receptor mimetics could lead to a decrease in side effects associated with anti-inflammatory drugs. EP2 is the primary PGE_2 receptor coupled to cAMP formation and stimulation of P4 production acting via activation of the protein kinase A signaling pathway [45]. In this study, we have examined the expression of the EP2 receptor. Western blot analysis showed the expression of EP2 receptor was induced by D-gal and was reversed by troxerutin. There still remains the possibility that other PGE_2 receptor signaling may be involved in this process. Further studies are in progress to be carried out.

The above results indicated that both oxidative damage and inflammatory damage occurred in the kidneys of D-gal-treated mice. Troxerutin has undergone numerous clinical trials in human subjects. Even with high doses, troxerutin had excellent safety and tolerability profiles [46]. As shown in Fig. 3, treatment with troxerutin on the D-gal-treated mice markedly increased antioxidant ability of SOD, CAT and GPx activities, compared to D-gal alone. These results suggested that the protective effect of troxerutin may be related to the increased ability of scavenging H_2O_2. In addition, Fig. 2 showed that troxerutin can significantly decrease the production of MDA induced by D-gal. The above results indicated that troxerutin could attenuate oxidative injury in the mouse kidney. In addition, our results in present study showed that troxerutin has the ability to inhibit the expression of iNOS and COX-2 in the kidneys of D-gal-treated mice. Our findings pointed that troxerutin firstly scavenges the ROS, which is induced by D-gal, and subsequently normalizes/inhibits excessive production of NO resulting from iNOS induction. Then, it results in reduction of the expression of COX-2. In other words, the normalization/inhibition of NO production by treatment of troxerutin brings about blockage of the inflammatory process. We found that troxerutin could inhibit the upregulation of NF-κB, which led to suppression of iNOS induction and inducible NO production, and then blocked the COX-2 induction. Furthermore, we examined the expression of the EP2

receptor. The results indicated that PGE2 exerts its effects through, at least in part, EP2 receptor [3,44,47].

In conclusion, troxerutin, a rutoside derivative, has effectively antioxidative and anti-inflammatory activities. Our present study showed that troxerutin could attenuate the renal injury induced by D-gal in mice (Fig. 1), through (1) elevating antioxidant enzymes activities and reducing MDA content, (2) inhibiting NF-κB upregulation and decreasing the expressions of iNOS and COX-2.

Acknowledgments

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