European Journal of Pharmacology 649 (2010) 382–389

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Endocrine Pharmacology

Tetrahydroxystilbene glucoside ameliorates diabetic nephropathy in rats: Involvement of SIRT1 and TGF-β1 pathway

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ARTICLE INFO

Article history:
Received 10 January 2010
Received in revised form 19 August 2010
Accepted 6 September 2010
Available online 18 September 2010

Keywords:
Diabetic nephropathy
Mesangial cell
SIRT1
TGF-β1
2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG)

ABSTRACT

Oxidative stress caused by hyperglycaemia is believed to be a major molecular mechanism underlying diabetic nephropathy. 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG), an active component extract from *Polygonum multiflorum* Thunb, exhibits antioxidative and anti-inflammatory effects. Possible protective mechanisms of TSG on diabetic nephropathy were investigated in rats and cultured rat mesangial cells. Total cholesterol and triglyceride levels of diabetic rats were clearly increased and these increases were diminished by treatment with TSG. Treatment of diabetic rats with TSG also significantly reduced blood urea nitrogen, creatinine, 24 h urinary protein levels, and kidney weight/body weight. The activities of superoxide dismutase and glutathione peroxidase in renal homogenate were increased markedly, whereas malonaldehyde levels were decreased significantly in TSG-treated diabetic rats. TSG dramatically inhibited diabetes-induced overexpression of TGF-β1 and COX-2, and restored the decrease of SIRT1 expression in diabetic rats. High glucose-induced overexpression of TGF-β1 in cultured mesangial cells was significantly inhibited, whereas the decease of SIRT1 expression was restored by pretreatment of TSG. Nicotinamide, the inhibitor of SIRT1, partially relieved the inhibitory effect of TSG on TGF-β1 expression under high glucose condition. These findings indicate that the protective mechanisms of TSG on diabetic nephropathy are involved in the alleviation of oxidative stress injury and overexpression of COX-2 and TGF-β1, partially via activation of SIRT1.

1. Introduction

Diabetic nephropathy is one of the most severe complications of diabetes mellitus and has become the largest cause of end-stage renal disease (Rossing, 2006). Overproduction of reactive oxygen species, a direct consequence of hyperglycemia, is usually attributed to the major factor in the development of diabetic nephropathy (Rosen et al., 2001). Reactive oxygen species mediates hyperglycemia-induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes such as transforming growth factor-β1 (TGF-β1) (Lee et al., 2003).

TGF-β1 is thought to play an important role in mediating the hypertrophic and fibrotic/sclerotic manifestations of diabetic nephropathy (Sharma and Ziyadeh, 1995). Up-regulation of TGF-β1 has been found in cell culture systems, experimental animal models, and human studies (Sharma and Ziyadeh, 1994; Wolf et al., 1992; Yamamoto et al., 1993). Previous studies have provided evidence that TGF-β1 mediates the accumulation of extracellular matrix molecules in mesangial cells and tubular cells (Han et al., 1999). Inhibition of TGF-β1 significantly reduced renal fibrosis and decreased the mRNA levels of key mediators of extracellular matrix deposition in the kidneys of db/db mouse (Petersen et al., 2008).

It has been reported that SIRT1, a class III histone deacetylase, inhibits TGF-β1 induced apoptosis in glomerular mesangial cells via Smad7 deacetylation (Kume et al., 2007). Overexpression of SIRT1 attenuates reactive oxygen species-induced apoptosis in mesangial cells and up-regulation of SIRT1 may provide a new strategy for preventing renal glomerular diseases (Kume et al., 2006; Tikoo et al., 2008). SIRT1 also increases the activity of manganese superoxide dismutase (SOD), a potent antioxidant enzyme, which in turn increases the cellular ability to detoxify reactive oxygen species (Brunet et al., 2004). Resveratrol (trans-3, 4′, 5-tri-hydroxystilbene), a natural potent activator of SIRT1, ameliorates diabetic nephropathy in the experimental animal model (Sharma et al., 2006). 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (TSG), one of the active polyphenolic components extracted from *Polygonum multiflorum* Thunb, protects hippocampus neuron from oxygen to glucose deprivation-induced injury via SIRT1 pathway(Wang et al., 2009). Previous studies have demonstrated that inhibition of COX-2 ameliorates renal injury in diabetic and hypertensive rats or in uninephrectomized diabetic rats

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doi:10.1016/j.ejphar.2010.09.004

(Cheng et al., 2002; Komers et al., 2007). In addition, TSG can also inhibit COX-2 enzyme activity and expression (Zhang et al., 2007).The polyphenolic structure of TSG is similar to that of resveratrol, which attract our interest to explore whether TSG could be used to treat diabetic nephropathy.

The objective of the present study was to investigate the protective effects and the mechanisms of TSG on diabetic nephropathy. In particular, we chose to analyze the effects of TSG on oxidative stress markers, inflammatory, SIRT1 and TGF-β1 expression in the kidney of diabetic rats. To further understand this knowledge, we also investigated the in vitro effects of TSG on the expression of TGF-β1 and SIRT1 in mesangial cells under high glucose condition. Our results present here point to a role for SIRT1/ TGF-β1 signaling in the protective effects exerted by TSG on diabetic nephropathy.

2. Materials and methods

2.1. Animals

The research was conducted in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. All experimental protocols were approved by the Review Committee for the Use of Human or Animal Subjects of Xianning University. Male Sprague–Dawley rats, approximately 220 ± 14 g, were obtained from the Center of Experimental Animal of Hubei Academy of Medical Sciences. These rats were housed in a temperature- and humidity-controlled environment, and they were allowed free access to tap water and standard rat chow.

2.2. Experimental protocols

Rats were administered with streptozotocin (Sigma Chemical Co. St. Louis, Mo., USA) as a single dose of 60 mg/kg intraperitoneal injection in citrate buffer. The control diabetic rats were received an injection of the buffer only. The levels of blood glucose were determined 72 h after injection of streptozotocin, and the diabetic rats were confirmed by blood glucose concentrations >16.7 mmol/l. Rats were randomly divided into four groups as follows: control group, untreated diabetic group, low dose of TSG-treated group(10 mg/kg), and high dose of TSG-treated group(20 mg/kg). Body weight and blood glucose levels were monitored regularly. After treatment with TSG for 8 weeks, 24 h urinary protein and blood samples were collected before rats were sacrificed. Kidneys were removed, decapsulated and weighed. Renal cortex tissues were harvested and kept in liquid nitrogen before tests. TSG (dissolved in distilled water, molecular weight: 406, purity above 98%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

2.3. Cell culture and treatment

The rat mesangial cells line (HBZY-1) was purchased from Center of Type Culture Collection, China. High glucose has been considered as an important stimulus to increase proliferation of mesangial cells. Mesangial cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 on culture plates with 10% fetal bovine serum-supplemented DMEM medium. All the subsequent procedures were carried out under these conditions. For the different experiments, mesangial cells were pretreated with a series of concentrations of TSG (1, 5, 25, 100 μM) for 48 h and then stimulated with 30 mM glucose for 24 h. In the Western blot experiments, mesangial cells were conducted as follows: Control (5.5 mM glucose), High glucose (30 mM glucose), TSG-treatment (30 mM glucose plus 100 μM TSG), and Mannitol (24.4 mM mannitol).

2.4. Biochemical and kidney weight/body weight analysis

Blood samples were collected for measurement of blood glucose, total cholesterol, triglyceride, creatinine, and blood urea nitrogen. Twenty-four hour urine of each rat was collected individually by metabolic cage to measure the 24 h urinary protein. All the measurements were conducted by using commercial kits (Jiancheng biomedical engineering Co. Ltd., Nanjing, China). The estimate of lipid peroxidation of the renal cortex homogenate was determined by measuring the formed malondialdehyde (MDA), which was commonly determined by the modified thiobarbituric acid method. SOD and glutathione peroxidase (GSH-Px) activities of the renal cortex homogenate were measured by using commercial kits following the protocols (Jiancheng biomedical engineering Co. Ltd., Nanjing, China).

2.5. Histopathological examination

After measurement of the renal weight, the kidney tissues were fixed in 10% neutral buffered formalin. Paraffin-embedded kidney tissues were cut into 3–4 μm. Subsequently, the representative sections were stained with hematoxylin and eosin for optical microscopy measurement. Renal pathologist carried out histopathological examinations in a blinded manner.

2.6. Cell viability assessments

Mesangial cells proliferation was examined by MTT assay. In live cells, mitochondrial enzymes have the capacity to transform MTT into insoluble formazan. Following incubation with various treatments, MTT solution (5 mg/ml) in DMEM medium was added to each well. Then, the medium was discarded and DMSO was added to solubilize the reaction product formazan by shaking for 5 min. After incubation at 37 °C for 4 h, the MTT solution was removed from the medium. Absorbance was detected at 570 nm using a microplate reader (ELx800, Bio-Tek, Winooski, VT, USA). Cell viability of control group not exposed to either high glucose or TSG was defined as 100%.

2.7. Western blot analysis

The renal cortex and mesangial cells were used for Western blot analysis. The procedures were processed according to our previous protocols with some minor modifications (Cai et al., 2008). Briefly, protein concentrations were determined using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). All samples were adjusted to equal protein content before analysis and then separated on 10% SDS-PAGE gels. After electrophoresis, proteins were transferred to a nitrocellulose membrane (transfer buffer: 25 mM Tris, 190 mM glycine, 20% methanol, 0.5% sodium dodecyl sulfate) by using a transfer cell system (Bio-Rad). After being blocked for two hours at room temperature in blocking buffer (5% nonfat milk in 20 mM Tris/HCl, pH 7.6, 140 mM NaCl, 0.5% Tween 20), membranes were incubated over night at 4 °C with the appropriate primary antibodies against TGF-β1, SIRT1, COX-2 and β-actin. Membranes were then washed (in 20 mM Tris/HCl, pH 7.6, 140 mM NaCl, 0.1% Tween 20) and incubated with a horseradish peroxidase-conjugated secondary antibody at room temperature for 50 min. Immunoblots were developed on films using the enhanced chemiluminescence technique (SuperSignal West Pico; Pierce). Band intensities were quantified using NIH ImageJ software and normalized to the quantity of β-actin in each sample lane. All assays were performed at least four times.

2.8. Statistics

Data are presented as means ± S.E.M. One-way ANOVA combined with post hoc analysis (LSD) was used for the statistical analysis by