Gene 591 (2016) 411–416

Contents lists available at ScienceDirect

Gene

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

Puerarin, isolated from *Pueraria lobata* (Willd.), protects against diabetic nephropathy by attenuating oxidative stress

Xiaohui Xu [b,1], Ni Zheng [c,1], Zhaoni Chen [b], Wansu Huang [b], Tao Liang [a,*], Hai Kuang [a,*]

[a] College of Stomatology of Guangxi Medical University, Nanning, Guangxi 530021, PR China
[b] Pharmaceutical College, Guangxi Medical University, Nanning, Guangxi 530021, PR China
[c] Shenzhen University, Shenzhen, Guangdong 518060, PR China

ARTICLE INFO

Article history:
Received 12 April 2016
Received in revised form 9 June 2016
Accepted 14 June 2016
Available online 16 June 2016

Keywords:
Puerarin
Diabetic nephropathy
Reactive oxygen species
Renal protection

ABSTRACT

In this study, we evaluated the effect of puerarin (PR) on diabetic nephropathy (DN) in streptozotocin (STZ)-induced diabetic mice. The fasting blood glucose (FBG), blood urea nitrogen (BUN) and serum creatinine (Scr), as well as 24-hour urine protein levels were effectively ameliorated in DN mice treated with PR (20, 40, 80 mg/kg/day). Furthermore, PR treatment markedly resulted in down-regulation of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and reactive oxygen species (ROS) in kidney. Interestingly, the activities of manganese superoxide dismutase (MnSOD) and catalase (CAT) were increased by PR. An improvement in kidney tissue damage could be observed after PR administration. Further ultrastructural investigation revealed a dramatically ameliorative effect of PR on mitochondrial damage. Meanwhile, the silent information regulator 1 (SIRT1), forkhead box protein O1 (FOXO1) and alpha subunit of peroxisome proliferators-activated receptor-gamma coactivator-1 (PGC-1α) expressions were significantly up-regulated at protein level by PR administration in renal cortex. However, the protein expression of nuclear-factor kappa B (NF-κB) was down-regulated in PR groups. Our present study demonstrates the hypoglycemic and renal protective effects of PR in DN mice, which support its anti-diabetic property. PR exerts its renal protection effect probably via the mechanism of attenuating SIRT1/FOXO1 pathway for renal protection.

1. Introduction

Diabetic nephropathy (DN) characterized with glomerular hypertrophy, basement membrane thickening and accumulation of extra cellular matrix (ECM) is one of the most severe complications of diabetes mellitus (DM). The prevalence of DN is increasing every year, which makes DN be the major cause of end-stage renal disease (ESRD) and death (Maezawa et al., 2015). Numerous studies have demonstrated that excessive ROS was produced by mitochondrial oxidative stress. Mitochondrial oxidative stress mediated signaling pathway may be the underlying mechanism of leading kidney tissue cells damage in DN (Chen, Chen et al., 2015).

It has reported that lots of Chinese herbal medicines (CHM) have therapeutic effects on diabetes and its complications with fewer side effects (Chung et al., 2015). And the underlying mechanism may be associated with SIRT1/FOXO1 signaling pathway (Zhu et al., 2014). The research of Tan KY et al. found that resveratrol, a SIRT1 activator, could attenuate mitochondrial malfunction in diabetic mice by scavenging ROS,thereby attenuating the process and minimizing the pancreatic and skeletal damage (Tan et al., 2011). Much of the research in diabetes has examined that the activity of SIRT1 was decreased both in the STZ-induced diabetic model and spontaneously type 2 diabetic KKAy mice. Once the expression of SIRT1 was up-regulated through transgenics, activator or energy restriction methods, an ameliorative effect on DN was observed (Kume et al., 2013). SIRT1/FOXO1 signaling pathway is closely involved in the progress of DN. Recent study has reported that the levels of MnSOD and CAT were enhanced by activating FOXO, which give rise to the melioration on mitochondrial damage and renal interstitial fibrosis in DN (Ji et al., 2014). It has been suggested that mitochondrial respiratory chain complex damage is associated with excessive levels of ROS under diabetic conditions, finally led to mitochondrial apoptosis (Ji et al., 2014). PGC-1α a key regulator on mitochondrial biogenesis could regulate the synthesis of mitochondrial respiratory chain complex (Cheng et al., 2009). The stimulation of SIRT1 on PGC-1α could efficiently prevent podocytes from damage. In the

Abbreviations: PR, puerarin; DN, diabetic nephropathy; STZ, streptozotocin; FBG, fasting blood glucose; BUN, blood urea nitrogen; Scr, serum creatinine; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α;; ROS, reactive oxygen species; MnSOD, manganese superoxide dismutase; CAT, catalase; SIRT1, silent information regulator 1; FOXO1, forkhead box protein O1; PGC-1α, peroxisome proliferators-activated receptor-gamma coactivator-1; NF-κB, nuclear-factor kappa B; ECM, extra cellular matrix; CHM, Chinese herbal medicines.

* Corresponding authors.
E-mail addresses: kqliangtao@126.com (T. Liang), kuanghai@hotmail.com (H. Kuang).

[1] First co-author; these authors contributed equally to this work.

http://dx.doi.org/10.1016/j.gene.2016.06.032
0378-1119/

SIRT1 gene knockout with high fat diet mice, the podocytes damage appears after a period of time and the ROS level is increased. This may indicate that the SIRT1 might stimulate the synthesis of PGC-1α through activating downstream pathway of FOXO1.

Puerarin (PR), one of active compounds of *Pueraria lobata* has been reported that PR intervention mediates hepatoprotective effects on non-alcoholic disorders and alcohol-induced adiposis hepatica (Li et al., 2013). In addition, PR has been promoted as a therapy for DN by reducing serum levels of TNF-α and IL-6 (Wang et al., 2014). Meanwhile, our previous study found that PR functioned as anti-diabetic activity through elevating insulin expression and maintaining metabolic homoeostasis in STZ-induced diabetic mice (Wu et al., 2013). Thus, we initiate our efforts to assess the potential underlying anti-diabetic nephropathy mechanism of PR by using DN mice induced by streptozotocin, which will provide an experimental basis for further mechanism research via SIRT1/FOXO1 on RMP.

2. Materials and methods

2.1. Chemicals

Puerarin preparation (purity > 99%) was provided by the Department of Pharmaceutical Chemistry, Guangxi Medical University (Nanning, China). The molecular structure of PR as showed in Fig. 1. Streptozotocin (STZ) was purchased from Sigma Co., Ltd. (Missouri, USA). FBG and blood chemistry detections were measured with the Roche ACCU-CHEK® Performa (Strip lot: 470664, Switzerland) and automatic biochemical analyzer (Hitachi Model 7100 Automatic Analyzer). Other required materials are outlined in the following sections.

2.2. Animals and drug administration

Healthy male C57BL/6 mice with 18–22 g of weight were obtained from the Experimental Animal Center of Guangxi Medical University (registration number SCXK 2010-0002). All mice were housed under controlled conditions with temperature of 25 ± 1 °C, relative humidity of 60 ± 5%, room air changes 12–18 times/h, and a 12 h light/dark cycle. All the animal protocols were approved by the institutional ethics committee of Guangxi Medical University (approval No.: 2012011121).

The mice were adaptively fed with standard rodent chow and water for a week and fasted for 12 h before treatment with STZ at 120 mg/kg of body weight via tail vein injection. 72 h later, the FBG and urinary albumin tests were conducted, and the mice with FBG ≥ 11.1 mmol/L and albuminuria were considered as DN mice. DN mice were assigned into 5 groups (n = 10, per group): DN model control group, metformin positive control group (200 mg/kg), low-, moderate-, and high-dosage PR groups (20, 40, 80 mg/kg). Meanwhile, number of 10 healthy mice was chosen as normal control group. And the drugs were intragastrically given to mice daily for 8 consecutive weeks.

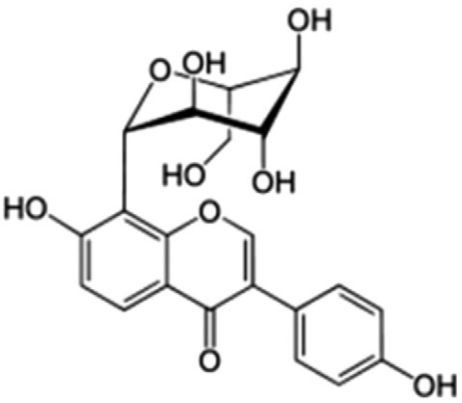

Fig. 1. The chemical structure of puerarin isolated from *P. lobata* (Willd.)

2.3. Collection of the blood and tissues

During the experiment, FBG was measured from the tail vein on 0, 4 and 8 weeks and 24-hour urine was collected using metabolic cages. The mice were sacrificed with sodium pentobarbital (150 mg/kg) after 8 weeks of PR treatment. The serum was collected from whole blood by centrifugation at 1300 ×*g* for 10 min. The renal samples were removed, fixed with 4% paraformaldehyde for histology assays and stored at −80 °C until further analysis.

2.4. Measurement of biochemical index

FBG was tested by blood glucose meter. BUN, Scr and 24-hour urine protein levels were analyzed using automatic biochemical analyzer (Hitachi Model 7100 Automatic Analyzer).

2.5. Measurements of IL-6, TNF-α, ROS, MnSOD and CAT levels in kidney tissue

The renal levels of IL-6 and TNF-α were analyzed using enzyme-linked immunosorbent assay (Elisa) kits (Boster Bio-Engineering Co., Ltd., Wuhan, China) following the kit instructions. The activities of MnSOD, CAT and ROS were detected using colorimetry methods (Beijing Yonghui Biological Technology Co., Ltd., Beijing, China).

2.6. Histopathological examination

The renal samples were embedded with paraffin after fixed with 4% paraformaldehyde for at least 24 h. The renal sections were performed with hematine-eosin (HE) staining following routine procedure and were observed under an optical microscope for histopathological examination.

2.7. Observation of pancreas ultrastructure

The removed renal samples were cut into small pieces (about 2 mm × 2 mm × 2 mm) and fixed in 2.5% pre-cooling glutaraldehyde immediately for 2 h under low temperature 0 °C. All the samples were post-fixed for 4 h with 1.5% osmium tetroxide, dehydrated, embedded and made into sections. Finally, the specimens were stained with 3% uranyl acetate/lead citrate and observed under a transmission electron microscopy (HITACHI H-7650).

2.8. Western blot analysis of SIRT1, FOXO1, PGC-1α and NF-κB

The separation of protein from renal tissue was performed using 10% sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) and transferred to a nitrocellulose membrane. And the membrane was immuno-blotted with primary antibodies against SIRT1, FOXO1, PGC-1α, NF-κB and β-actin overnight at 4 °C. A goat anti-rabbit and/or goat anti-mouse horseradish peroxidase conjugated secondary IgG (Boster Biotechnology) was used as the secondary antibody and the filter images were performed with a gel image analysis system (UVP) after staining with diaminobenzidine. Signals of band densities were determined by Scion image software (Scion Corp., Frederick, MD) and were normalized to β-actin after quantitative estimation.

2.9. Statistical analysis

All data are expressed as the mean ± S.E. and were analyzed statistically using SPSS 16.0 software (SPSS Inc., USA). Differences between groups were evaluated using one-way analysis of variance (ANOVA). Difference between groups were considered statistically significance at $P < 0.05$.