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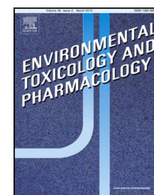

Inhibitory effects and mechanism of 25-OH-PPD on glomerular mesangial cell proliferation induced by high glucose

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ABSTRACT

Objective: To investigate the protective effects and potential mechanism of the compound 25-OH-PPD (PPD) on the glomerular mesangial cells (GMC) under high glucose condition.
Methods: The hypertrophic GMC cells were established by DMEM containing glucose and randomly divided into five groups, including the normal control group (Control), the high glucose model group (HG, 25 mmol L^{-1}), the PPD low dose group (1 μmol L^{-1}, PPD-L), the PPD middle dose group (5 μmol L^{-1}, PPD −M) and the PPD high dose group (10 μmol L^{-1}, UCN-H). The GMC were incubated for 48 h under different treatment factors. Total protein content was determined by Lowry method. The diameter of the single GMC and volume were measured by computer photograph analysis system. The GMC cell viability was analyzed by MTT assay. The level of malondialdehyde (MDA), the content of glutathione (GSH) and superoxide dismutase (SOD) activity were measured by ELISA. $[Ca^{2+}]_i$ transient was measured by Till image system and by cell-loading Fura-2/AM. The expression of COX-1 and COX-2 were also determined using ELISA method.
Results: The viability of GMC and the total protein content were decreased in HG group, different dosage PPD group could increase these indexes ($P < 0.05$). The level of MDA was increased, the content of GSH and SOD was decreased in HG group, while PPD could reduce the MDA and enhance GSH and SOD ($P < 0.05$). Following treatment with different dosage (PPD-L, PPD-M or PPD-H), the $[Ca^{2+}]_i$ transient was reduced ($P < 0.05$ or $P < 0.01$). Moreover, the expression of COX-1 was decreased while COX-2 expression was increased in different dosage PPD groups.
Conclusion: The protective effects of PPD on GMC from HG-induced hypertrophy may be associated with the inhibition of $[Ca^{2+}]_i$ transient and decreasing expression of COX-1 *via* the oxidative-stress injure pathway.

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1. Introduction

As a part of systemic micro-vascular disease, diabetic nephropathy(DN) that develops in approximate 30%–35% patients with diabetes is the primary cause of chronic renal failure. According to the statistics from European Renal Dialysis and Transplant Association, DN is one of the major causes for the death of diabetics, accounting for more than one third of all patients with chronic renal failure (Wang et al., 2015; Bakris et al., 2015). As DN is reversible in its earlier stage, prevention and early treatment are of valuable, especially for exploring comprehensive treatments with traditional Chinese medicine (Robles et al., 2015; Badal and Danesh, 2015).

Compared with western pharmaceutical preparations, traditional Chinese medicine embraces characters of "multiple targets and multiple effects". For example, panax notoginseng saponins and rooted salvia which can promote blood circulation, remove blood stasis, resist oxidation, regulate metabolic disturbances and curb the formation of advanced glycation end-products (AGEs) in addition to improving renal microcirculation, anticoagulation and inhibiting platelet aggregation, have been shown to reduce urine protein and postpone the process of renal failure (Hong et al., 1993). Pseudo-ginseng, the dried root of panax notoginseng (BurK) F.H.Chen, is honored as a precious pearl in the treasure house of traditional Chinese medicine. It says in A Supplement to Compendium of Materia Medica of Qing dynasty that "Ginseng is the best in tonifying Qi, while pseudo-ginseng is the best in enrich-

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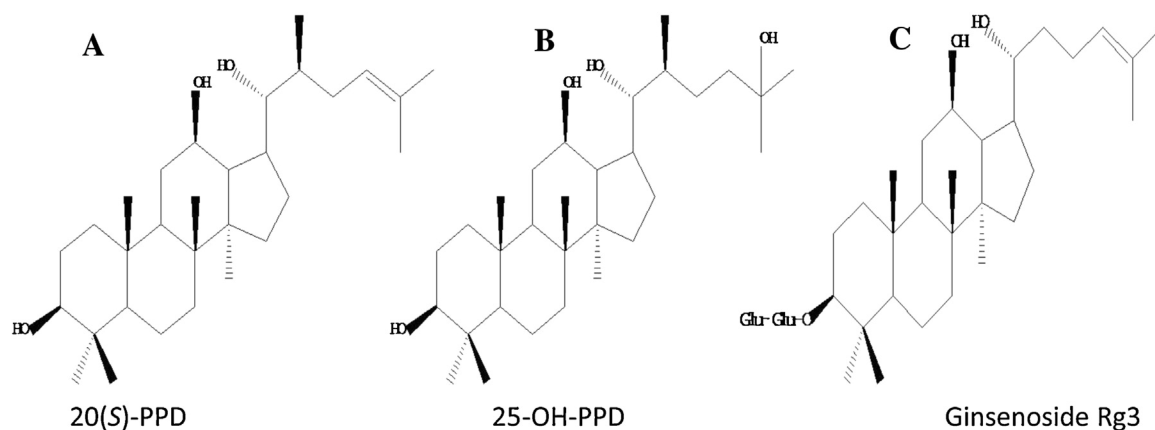

Fig. 1. The chemical structures of 20(*S*)- PPD (1A), 25-OH-PPD (1B) and its' derivative Rg3 (1C).

ing the blood; as the latter has the similar taste and functions with that of ginseng, thus it is most valuable in traditional Chinese medicine." The medicinal parts of pseudo-ginseng include the root, stems and leaves and its flower. Modern pharmacological studies have shown that the root of pseudo-ginseng can protect diabetes patients from renal injuries through many pathways such as regulating hyperglycemia, hyperlipidemia, hyperviscosity and hypertension (Shukla et al., 1992).The main ingredients of the pseudo-ginseng root is panax notoginseng saponins (PNS) which can effectively improve the renal microcirculation, reduce urine protein excretion and the formation of immune complex, decrease injuries in blood vessels, kidney and platelet activation, lower the platelet adhesiveness and the blood viscosity, shorten the platelet recovery, quicken blood current velocity, and protect the renal function (Zhang et al., 2014). Recent studies have shown that notoginseng leaf triterpenes, the active ingredient of the stem and leaves of pseudo-ginseng, is also dammarane triterpenoid saponin with similar pharmaceutical functions with PNS, which is able to ease pain and resist coagulation and inflammation, *etc.* (Zhao et al., 2015). Notoginseng leaf triterpenes can effectively lower levels of total cholesterol and the triglyceride in the serum of rats and quails which are models of high fat (Chen et al., 2015). 25-OH-PPD (PPD), a new terpene saponin separated and identified from the stem and leaves of pseudo-ginseng, has evident reported (Wu et al., 2015) 25-OH-PPD (PPD) had anti-tumor activity also used in diabetes studies. The present study aimed to investigate the inhibitory effects and mechanism of PPD on glomerular mesangial cells proliferation that was caused by high glucose level (Fig. 1).

2. Materials and methods

2.1. Chemicals and reagents

The test compound 25-OH-PPD (PPD, (>95% pure)) was kindly provided by Dr. Ruiwen Zhang (Texas Tech University Health Sciences Center, USA) (Wang et al., 2007; He et al., 2014). Rat HBZY-1 mesangial cells were obtained from the Cell Resource Center, School of Basic Medicine Peking Union Medical College (Beijing, China). Dulbecco's modified Eagle medium (DMEM), trypsin and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Beijing (Beijing Bio-chemical Reagent Company). Assay kits for Malondialdehyde (MDA), super oxide dismutase (SOD) and glutathione peroxidase (GSH-Px) was purchased from Shanghai (Beyotime Institute of Biotechnology). Anti-cyclooxygenase-1/2 (COX-1/2) antibodies were purchased from Shanghai (Beyotime Institute of Biotechnology).

2.2. Cell culture and treatment

GMC-HBZY-1 cells were cultured in DMEM containing 10% FBS and penicillin/streptomycin (100 and 100 μg/mL) at 37 °C in 5% CO_2-humidified atmosphere. After being detached with 0.25% trypsin, cells were plated in six-well plates to grow until reaching a confluence of 70–80%, and cells were treated with DMSO. In different experiments, cells were randomly divided into the following groups: (1) Normal group: the cells were cultured in normal glucose medium (5.56mmol L^{-1}); (2) HG group: the cells were cultured in high glucose medium (HG, 25mmol L^{-1}); (3) PPD-L group: the cells were cultured in HG medium (25mmol L^{-1}) + 25-OH-PPD (low dose PPD, 1 μmol L^{-1}); (4) PPD-M group: the cells were cultured in HG medium (25mmol L-1) + 25-OH-PPD (middle dose PPD, 5 μmol L^{-1}); (5) PPD-H group: the cells were cultured in HG medium (25mmol L^{-1}) + 25-OH-PPD (high dose PPD, 10 μmol L^{-1}). 30 min following these treatments, cells were treated with high glucose and harvested at 48 h post incubation for analysis.

2.3. Measurement of cell's volume

After HBZY-1 cells were cultured in the 24-well plate for 48 h, they would be divided randomly into five groups with six wells as a team when the cells were in a good state. Then the medium was changed and medication was added. In another 48 h' period, PBS solution (NaCl 8.00 g L^{-1}, KCl 0.20 g L^{-1}, $Na_2HPO_4 \cdot 12H_2O$ 3.489 g L^{-1}, KH_2PO_4 0.20 g L^{-1}, pH = 7.4) was employed to quickly rinse the cell-covered culturing-wells three times, and then 0.3 ml parenzyme (1 g L^{-1}, pH = 7.2) would be added to each well before the wells were put into the incubator. The incubation lasted for half an hour in a 37 °C incubator and stopped by adding 10% fetal bovine serum (0.2 ml/well). Digested cells were collected and measured on an inverted microscope. For measurements, four or five fields were randomly chosen from 16 or 20 fields in the direction of random table and observed under high power microscope (×400), and 80 individual cell areas were calculated by CIAS Daheng computer photograph analysis system, then the volume formula of sphere was used to calculate the volume of the cell.

2.4. Cytotoxicity assay

GMC-HBZY-1 cell viability was tested by MTT Cell Proliferation and Cytotoxicity Assay Kit. Briefly, 48 h later since the cell was chemicalized, the cells were inoculated into the 96-well plate, MTT (5g L^{-1}) 20 μl was added and then the plate was put into the incubator at the temperature of 37 °C for four hours. Then the culture medium was removed before adding DMSO 150 μl to dissolve

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