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The anti-inflammation effect of Moutan Cortex on advanced glycation end products-induced rat mesangial cells dysfunction and High-glucose–fat diet and streptozotocin-induced diabetic nephropathy rats

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ABSTRACT

Ethnopharmacological relevance: Moutan Cortex (MC, family: *Paeonia suffruticosa* Andr.) is a well-known traditional herbal medicine that has been shown to hold a protective effect on inflammation in several diseases. However, its anti-inflammatory activity on diabetic nephropathy (DN) has been less reported. Q9 The present study was conducted to evaluate the potential attenuation activities of MC on inflammation in AGEs-induced rat mesangial cells dysfunction and high-glucose–fat diet and streptozotocin (STZ)-induced DN rats and explore the possible mechanism underlying its DN effect.

Materials and methods: The inflammation in mesangial cells (HBZY-1) was induced by 200 μg/ml advanced glycation end products (AGEs). DN rats model was established by an administration high-glucose–fat diet and an intraperitoneal injection of STZ (30 mg/kg). Interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) level in cell supernatant and rats serum were detected by appropriate kits. A co-culture system of mesangial cells and macrophages was performed to evaluate the migration of macrophages. Immunohistochemical assay was applied to examine transforming growth factor beta1 (TGF-β1), IL-6, MCP-1 and intercellular adhesion molecule-1 (ICAM-1) expression in kidney tissues of rats. Furthermore, western blot analysis was carried out to examine TGF-β1, IL-6, MCP-1, ICAM-1 and RAGE protein expressions in mesangial cells.

Results: Pretreatment with MC could significantly inhibit AGEs-induced migration of macrophages in the co-culture system of mesangial cell and macrophage. MC could decrease IL-6 and MCP-1 levels in serum of DN rats in a dose-dependent manner. Furthermore, MC also improved the blood glucose, serum creatinine and urine protein levels. Both immunocytochemistry analysis and western blot analysis showed that MC decreased significantly the over-expression of IL-6, MCP-1, TGF-β1, ICAM-1 and RAGE in mesangial cells or kidney tissues. Additionally, the protein expression of proinflammatory cytokine could also be down-regulated by the pretreatment of RAGE-Ab (5 μg/ml).

Conclusion: These findings indicated that the extract of MC had an amelioration activity on the inflammation in AGEs-induced mesangial cells dysfunction and high-glucose–fat diet and STZ-induced DN rats. The protective effect might be associated with the intervention of MC via target of RAGE. These findings suggested that MC might be a benefit agent for the prevention and treatment of DN.

Abbreviations: AGEs, advanced glycation end products; MC, Moutan Cortex; DN, diabetic nephropathy; STZ, streptozotocin; AG, aminoguanidine; TGF-β1, transforming growth factor beta1; PBS, phosphate buffered saline; ROS, reactive oxygen species; FBS, fetal bovine serum; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; RAGE, receptor for AGEs; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; ICAM-1, intercellular adhesion molecule-1; OD, optical density; SD, standard deviation; OS, oxidative stress

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1. Introduction

Diabetic nephropathy (DN), a common microvascular complication in diabetic patients causing end-stage renal disease, has led to a high morbidity and mortality both in teenagers and elderly persons throughout the word (Fox et al., 2005). There is a growing body of evidence to demonstrate the fact that the glucose metabolism disorders can contribute to the pathogenesis of DN (Hendig et al., 2008). More importantly, studies in recent years have shown that inflammation plays a role in the progression of microvascular diabetic complications such as DN (Navarro and Mora, 2005; Jeffcoate et al., 2005). It is worthy of note that there is a key relationship between DN and inflammatory cytokines. In addition, the effort to control the inflammatory pathways can slow the progression of DN (Navarro et al., 2003). Accumulating evidences have shown that interleukin-6 (IL-6) (Cheng et al., 2011), monocyte chemoattractant protein-1 (MCP-1) (Royall et al., 1989; Melcion et al., 1982), transforming growth factor-β (TGF-β) (Chen et al., 2003) and intercellular adhesion molecule-1 (ICAM-1) are all important inflammatory biomarkers involving in the development of DN (Cha et al., 2013).

Traditional Chinese medicine has been used for the treatment of DN for centuries. A lot of attention has been paid to them as the new therapeutic agents for DN (Poon et al., 2011). As a well-known traditional herbal medicine from the root bark of *Paeonia suffruticosa* Andr., Moutan Cortex (MC) has shown its great effects on analgesic, sedative and anti-microbial properties (JJiang et al., 2007). Interestingly, most researchers have focused on the anti-inflammatory activity of MC in recent years in rat synoviocytes (Wu and Gu, 2009) and LPS-activated raw 264.7 cells (Chun et al., 2007). However, anti-inflammatory activity of MC on potential attenuation of AGEs-induced rat mesengial cell disfunction and effect of MC on DN and its mechanism of action have not been investigated yet. Therefore, current study was proposed on investigation of possible mechanism of action in anti-inflammatory activity of MC on DN by using either *in vitro* or *in vivo* methods and AGEs-induced mesengial cell/macrophage co-culture method and high-fat/carbonhydrate diet fed and streptozotocin treated rat model were used for that purpose. It was aimed to identify possible anti-inflammatory mechanisms of action of MC underlying DN.

2. Materials and methods

2.1. Chemicals and drugs

Moutan Cortex (MC), derived from the roots of *Paeonia suffruticosa* Andr. (Batch No. 20120415), came from Anhui Huqiao Chinese Medicine Technology Co., Ltd. (Tongling, Anhui Province). The pharmaceutical botany of the medicinal material was identified by Prof. Dekang Wu in Nanjing University of Chinese Medicine. Rat HBZY-1 mesangial cell line was provided by Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). STZ, aminoguanidine (AG), bovine serum albumin (BSA) and D-glucose were provided by Sigma (St. Louis, MO, USA). Glucose measurement, creatinine determination, urinary protein quantification kits were provided by Nanjing Jianchen Bioengineering Institute (Nanjing, China). RAGE (Receptor for AGEs), TGF-β1, IL-6, MCP-1, ICAM-1 antibodies were from Boster (Wuhan, PR China). IL-6 and MCP-1 ELISA kits were purchased from KeyGEN Biotech. Co., LTD. (Nanjing, China). In addition, basal DMEM medium and fetal bovine serum (FBS) were provided by Gibco. HPLC-grade acetonitrile was purchased from Burdick & Jackson (Muskegon, MI). HPLC-grade water was obtained using a water purification system (Milli-Q Reagent Water System, MA, USA). Other chemicals for HPLC were of analytical reagent grade and from commercial source.

2.2. Preparation of AGEs

The preparation of advanced glycation end products (AGEs) was followed according to the previous description (Li et al., 2012). 5 g of BSA and 9 g of D-glucose were dissolved in 100 ml phosphate buffered saline (PBS) (0.2 M, pH 7.4). Then all the solution was passed through 0.22 μm microporous membrane filter, and incubated under sterile conditions for 3 months in 5% CO_2 at 37 °C. The brown reaction mixture was dialyzed against 0.01 M PBS overnight to remove unincorporated glucoses and low molecular reactants. AGEs were stored at −4 °C until used. Finally, the identification of AGEs was determined at excitation 370 nm and emission 440 nm in a Gemini EM fluorescence microplate reader (Molecular Devices, USA). The content of AGEs was determined by AGEs ELISA kit.

2.3. Preparation of MC

MC (150 g) was extracted with 500 ml of 75% (V/V) ethanol for 1 h twice. All the extracts were combined together to recycle the ethanol. The supernatant was obtained by centrifuging at the speed of 5000 rmp/min for 15 min. The extracts, obtained by evaporation in vacuum at 56 °C, were stored at −4 °C until needed.

2.4. Cell culture

HBZY-1 rat mesengial cells were grown in plastic cell culture dishes in 5% CO_2 at 37 °C, and maintained in low-glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% FBS (Gibco) according to the previous description (Kagami et al., 2004). To obtain sufficient nutrition for cells, all medium should be replaced every 2 days. Cells were used for the experiment to 24-well plates at a generating of 80%–90% confluent layer.

2.5. Animal model

All animal experiments procedures were in accordance with national and international laws for the use and care of laboratory animals. In the present study, male SD rats weighing 180–220 g were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, PR China), and maintained at a temperature of 25 °C at the relative humidity of 45%. The animal model method was as follows: rats for the control group ($n=6$/group) were fed with rat chow and water *ad lib* followed with the injection of 0.1 M citrate buffer pH 4.5. In DN group, rats were treated with the high-glucose–fat diet followed by a single intraperitoneal injection of STZ (30 mg/kg in 0.1 M citrate buffer pH 4.5) (Zhong et al., 2009). DN was confirmed by BG ≥ 12 mmol/L and urine protein ≥ 20 mg/24 h after being fasted for 48 h. DN rats were randomly divided into 5 groups: the model group (DN), positive control groups (AG of 0.1 g/kg), high dose group (MC of 5 g/kg), medium dose group (MC of 2.5 g/kg) and low dose group (MC of 1.25 g/kg). Normal saline was used for control and model groups, whereas AG and MC of different concentrations were used for relative groups via oral administration for one month.

2.6. Chromatographic conditions and equipment

The extract of MC was examined by Agilent 1200 series HPLC instrument (Agilent Technologies, Santa Clara, CA, USA). The sample was separated on an Agilent Eclipse TC-C_{18} column (4.6 mm × 150 mm, 5 μm). The mobile phase gradient conditions

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