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Original article

6-Shogaol ameliorates diabetic nephropathy through anti-inflammatory, hyperlipidemic, anti-oxidative activity in db/db mice

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ABSTRACT

The prevalence of type 2 diabetes mellitus has been increasing worldwide and more than two thirds of the patients may develop diabetic nephropathy (DN). However, the efficiency of existing approaches on DN progression is limited. 6-Shogaol (6-SG), a major dehydrated derivative of gingerols, possesses various biological properties. The present study was designed to evaluate the possible effects of 6-SG on DN in db/db mice and to investigate the mechanisms. We revealed that 6-SG reduced the levels of fasting blood glucose, serum insulin, C-peptide, glycosylated hemoglobin A1c, and systolic blood pressure. 6-SG decreased the levels of blood urea nitrogen (BUN), serum creatinine, urinary albumin content and albumin/creatinine ratio (ACR), ameliorated the pathological injuries of kidneys, reduced the surface area of Bowman's capsule, Bowman's space, glomerular tuft, and decreased the expression of collagen IV and fibronectin in kidneys of db/db mice. The high levels of systemic and renal triglyceride and cholesterol were decreased by 6-SG. Moreover, 6-SG exhibited anti-inflammatory effects, as reflected by reduction of tumor necrosis factor α (TNFα), monocyte chemotactic protein-1 (MCP-1), and IL-6 levels in circulation and kidneys, and decrease of NF-κB expression. Furthermore, 6-SG also inhibited oxidative stress and restored the expression of NF-E2-related factor 2 (Nrf2) in kidneys of db/db mice. In conclusion, we have demonstrated that 6-SG exhibits anti-diabetic and renal protective effects against DN, in which effect the anti-inflammatory, hyperlipidemic, anti-oxidative activities may be involved. Overall, 6-SG could be a promising therapeutic treatment to ameliorate diabetes and the development of DN.

1. Introduction

In the last decades, the prevalence of type 2 diabetes mellitus (T2DM) has been increasing worldwide and has reached globally epidemic proportions [1,2]. It is proposed that 40% T2DM patients may develop diabetic nephropathy (DN) [3]. DN is the major cause of end-stage renal disease (ESRD) [4]. The characteristics of DN include the accumulation of extracellular matrix (ECM) proteins and an irreversible decline in renal function [5]. The molecular mechanisms underlying DN are still not clear, and there are few effective therapies [6]. To date, the main methods for controlling DN are implemented by decreasing blood glucose levels and lowering hypertension through blockage of the renin-angiotensin system [7,8]. However, the efficiency of these approaches on DN progression and the development of ESRD is limited [9]. Thus, it is urgent to find novel therapies that may protect against DN.

Ginger (Zingiber officinale Roscoe, Zingiberacae) is a well known herb that has been widely used as an herbal medicine for various ailments for centuries. Gingerols is one of the major biologically active components extracted from ginger. Shogaols, pungent agents, is an important class of dehydrated derivatives of gingerols. 6-Shogaol (6-SG) has been reported to possess various biological effects, including anti-inflammatory [10], anti-diabetic [11], anti-tumor [12,13], anti-oxidative [14], neuroprotective [15], anti-amyloidogenic [16] activities. Ginger extracts have been shown to lower blood glucose level, restore the total carbohydrates, pyruvate, glycogen, and total protein in kidney tissue, regenerate tubules, restore glomeruli, and reduce fatty infiltration in STZ-induced diabetic rats [17,18]. However, the effect of 6-SG on DN is not known.

The present study was designed to evaluate the possible effect of 6-SG on DN in db/db mice and to investigate the mechanisms. We found that 6-SG could attenuate the glucose metabolic abnormalities and ameliorate the renal structural and functional injuries. 6-SG exhibited anti-inflammatory, hyperlipidemic, anti-oxidative activities which may contribute to the protective effect of 6-SG against DN.

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2. Materials and methods

2.1. Reagents

6-gingerol (G1046, > 98%) were obtained from Sigma-Aldrich Inc. (Saint Louis, MO, USA). P65 NF-κB antibody was purchased from Cell Signaling Biotechnology (CST, USA). β-actin and Nrf2 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Secondary antibodies were obtained from Thermofisher Scientific Biotechnology.

2.2. Animals and treatment

The Institutional Animal Care and Use Committee of The First Affiliated Hospital of Xinxiang Medical University and approved all animal procedures and experimental protocols. All animal welfare and experimental procedures adhered strictly to the Guide for the Care and Use of Laboratory Animals. 10 male db/m mice (wild-type) and 30 male C57BL/KsJ db/db obese mice were purchased from Model Animal Research Center of Nanjing University. The animals were housed in individual cages under specific pathogen-free (SPF) conditions in an air-conditioned animal facility at 23 ◦C on a 12-h light/dark cycle and were maintained with free access to water and food.

The db/db mice were 9-week-old with the blood glucose level > 11.1 mM. The db/db mice were allocated into three groups, with 10 mice in each group: db/db (DMSO in saline); 6-SG (25 mg/kg/day); 6-SG (50 mg/kg/day). 6-SG was dissolved in DMSO, diluted in saline solution and injected intraperitoneally. Mice in control and diabetes group were administered with vehicle only. The animal experiments were performed for a period of 12 weeks. In the end, blood pressures were measured on conscious acclimatized mice using tail cuff method. Blood samples were collected and kidney tissues were harvested with a part snap frozen in liquid nitrogen and another part fixed with 10% phosphate-buffered saline (PBS)-buffered formalin, processed, and embedded in paraffin.

2.3. Renal histopathology

After the treatment, renal cortex tissues were cut, washed with PBS, stored in 10% PBS-buffered formalin, and then processed and embedded in paraffin. Tissues were prepared as sections 4 μm in thickness and stained with hematoxylin-and-eosin. Tubular and glomerular damage was examined.

2.4. Morphometric analysis

The cross-sectional areas of the glomeruli and glomeruli capillary tufts were measured using the SIS automatic image analysing system (Software Imaging System GmbH, Münster) as previously described [19]. Capsular space was calculated as the difference between these two values. The thickness of the basement membranes of the Bowman's capsule and of glomerular capillary endothelium was measured at eight randomly chosen points.

2.5. Measurement of biochemical parameters

After 12 h fasting, the blood glucose level was measured through the venous blood from the tail vein using a glucometer (Roche, Mannheim, Germany). The blood and urine samples were collected. Kidney tissues were homogenized for further biochemical analysis. Glycated hemoglobin (HbA1c) was measured using the in2it A1C system (Bio-Rad, Hercules, CA). Serum and kidney levels of triglyceride and cholesterol and kidney levels of TBARS, GSH and GSSG were determined using commercial assay kits (Nanjing Jiancheng Company, Shanghai, China). To collect the morning spot urine samples, animals were placed in metabolic cages at the beginning of the light cycle and kept for 2 h with only water but without food. To obtain the 24 urine samples, animals were placed in metabolic cages at the beginning of the light cycle and kept for 24 h with free access to water and standard laboratory diet. Levels of creatinine and blood urea nitrogen (BUN) were determined using a Cobas®C311 Autoanalyzer (Roche Diagnostics, Indianapolis, USA) as per the manufacturer's protocols. Urinary albumin concentration was determined using an ELISA kit obtained from Bio-Medical Assays (Beijing, China). Levels of insulin, glucagon, C-reactive protein, C-Peptide, TNFα, MCP-1, and IL-6 were determined using ELISA kits obtained from Cusabio Technology (Wuhan, China). The lipid hydroperoxides (LPOs) in kidney homogenates were determined using a LPO assay kit (Cayman Chemical, USA).

2.6. Real-time quantitative PCR analysis

The kidney tissues were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, USA), and total RNA was extracted using the RNeasy mini kit (Qiagen, Germany) according to the manufacturer's instructions. The RNA was quantified using a Nanodrop ND-1000 spectrophotometer, and first-strand complimentary DNA (cDNA) was synthesized using 1 μg total RNA by the use of reverse transcription kit (Life Technologies, NY, USA) following the manufacturer's protocols. cDNAs were quantified by PCR using SYBR Green real-time PCR SuperMix kit (TaKaRa, Dalian, China) according to the manufacturer's instructions. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. Amplification reactions were performed on the CFX96 Real-Time System (Bio-Rad Laboratories, Inc.) as follows: one cycle of 94°C for 2 min, 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, followed by one cycle of 72°C for 7 min. The expression level was normalized to GAPDH and results were expressed as fold change relative to control group.

2.7. Western blot analysis

Kidney tissues were homogenized in lysis buffer (10 mM Tris–HCl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 1 μM dithiothreitol (DTT), proteinase inhibitor cocktail and phosphatase inhibitor). Samples equal to 20 μg protein were electrophoresed on a 10% SDS-PAGE gel. After that, proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% nonfat milk, the membranes were incubated with primary antibodies (P65: 1: 1000; Nrf2: 1: 500) at 4 °C overnight. After washing with TBST, the membranes were subsequently incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:5000 dilution) (Thermofisher Scientific, USA) for 60 min at 37 °C. The bands were visualized using enhanced chemiluminescence (ECL) reagents (Thermofisher Scientific, USA). β-actin was used as an internal control. Then, the relative amounts of the proteins were analyzed using Quantity ONE software and normalized to their respective controls.

2.8. Statistical analysis

The data were expressed as the mean ± standard error of the mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests using SPSS 16.0 software (SPSS Inc., Chicago, USA). Results were considered to be statistically significant when p value was less than 0.05.

3. Results

3.1. 6-SG improves diabetes in db/db mice

We assessed the effect of 6-SG on the glucose metabolic activities in db/db mice. The results showed that, as expected, the body weights of db/db mice were significantly higher than that of wild type mice and the administration of 6-SG significantly reduced the body weights of