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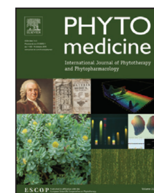

Original article

Protodioscin ameliorates fructose-induced renal injury via inhibition of the mitogen activated protein kinase pathway

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ABSTRACT

Background: High dietary fructose can cause metabolic syndrome and renal injury.
Purpose: The effects of protodioscin on metabolic syndrome and renal injury were investigated in mice receiving high-dose fructose.
Methods: Mice received 30% (w/v) fructose in water and standard chow for 6 weeks to induce metabolic syndrome and were divided into four groups to receive carboxymethylcellulose sodium, allopurinol (5 mg/kg) and protodioscin (5 and 10 mg/kg) continuously for 6 weeks, respectively. The glucose intolerance, serum uric acid (UA), blood urea nitrogen (BUN), creatinine (Cr), total cholesterol (TC), triglyceride (TG), interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined.
Results: Protodioscin significantly improved glucose intolerance and reduced the levels of serum UA, BUN, Cr, TC and TG. Histological examinations showed that protodioscin ameliorated glomerular and tubular pathological changes. Protodioscin significantly reduced renal concentrations of IL-1β, IL-6 and TNF-α by inhibiting the activation of nuclear factor-κB, c-Jun N-terminal kinase, p38 mitogen-activated protein kinase and extracellular signal-regulated kinase. In addition, the effect of protodioscin on the mitogen activated protein kinases (MAPK) pathway was examined.
Conclusion: Taken together, protodioscin is a potential drug candidate for high dietary fructose-induced metabolic syndrome and renal injury.

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Introduction

High-fructose corn syrup was introduced in the late 1960s, and since then, fructose consumption has increased markedly (Malik et al., 2006). Unlike other sugars, approximately 70% of fructose is taken up by the liver and the kidney (Aoyama et al., 2012). Epidemiological, clinical and experimental studies have proved that high dietary fructose may cause metabolic syndrome, such as dyslipidemia, insulin resistance, hyperglycemia, hypertension, hyperuricaemia and cardiovascular disease (Aoyama et al., 2012; Stanhope and Havel, 2010; Zawiasa and Nowicki, 2013). Metabolic syndrome is a constellation of risk factors of chronic kidney disease (CKD) and is a serious public health problem. Increasing research supports the view that insulin resistance induced by high dietary fructose, can cause hyperuricaemia and hyperglycaemia. The increase of both serum uric acid (UA) and blood glucose levels leads to renal injury through multiple avenues (Ma et al., 2015). UA has been suggested as an independent predictor of CKD, which can cause renal endothelial dysfunction, glomerular hypertension and cortical vasoconstriction (Chen et al., 2013). Previous evidence indicates that hyperglycaemia can activate transforming growth factor-β1 (TGF-β1) and lead to overexpression of inflammatory cytokines, subsequently inducing changes in the renal structure and function (Kajitani et al., 2010; Qi et al., 2008). Currently, only a few drugs are effective against CKD; therefore, there is a need to search for safe and effective drugs against CKD.

Dioscoreae rhizome (DR, Shan-yao in Chinese), the rhizome of *Dioscorea oppositifolia* L. (Dioscoreaceae) is an important food in

Abbreviations: Allo, allopurinol; BUN, blood urea nitrogen; CKD, chronic kidney disease; CMC-Na, carboxymethylcellulose sodium; Cr, creatinine; Dio, diosgenin; DR, Dioscoreae rhizome; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HOMA, homeostatic model assessment; IκBα, inhibitor of NF-κB-α; IL-1β, interleukin-1β; IL-6, interleukin-6; JNK, c-Jun N-terminal kinases; NF-κBP65, nuclear transcription factor-κB P65; OGTT, oral glucose tolerance test; p38 MAPK, protein-38 mitogen activated protein kinases; TC, total cholesterol; TG, triglyceride; TGF-β1, transforming growth factor-β1; UA, uric acid.

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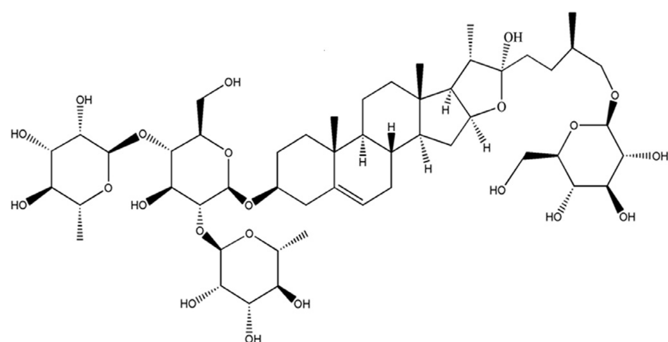

Fig. 1. Chemical structure of protodioscin.

China. DR has also been used in traditional Chinese medicine for centuries for invigorating the spleen, stomach and kidney (Shujun et al., 2006). Pharmacological studies have demonstrated the therapeutic effects of DR in numerous diseases, such as cardiovascular disorders, cancers, diabetes, neurodegenerative disorders, allergic diseases and amelioration of menopausal symptoms (Huang et al., 2011; Lee et al., 2002; McAnuff-Harding et al., 2006; Zhao et al., 2005). Protodioscin (Fig. 1), a major steroidal saponin in DR, has been shown to exhibit multiple biological actions, such as anti-hyperlipidemia, anti-cancer, sexual effects and cardiovascular properties (Gauthaman et al., 2003; He et al., 2006; Hu and Yao, 2002; Wang et al., 2010; Zhang et al., 2016).

In regards to the pathogenesis of renal injury induced by high dietary fructose and the pharmacological actions of protodioscin, we hypothesized that protodioscin has a beneficial effect in renal injury. The present study investigated the effects of protodioscin on metabolic syndrome and renal injury in mice treated with high-dose fructose. To elucidate its potential mechanism of action, the effect of protodioscin on the mitogen activated protein kinases (MAPK) pathway was examined.

Materials and methods

Chemicals and reagents

Protodioscin (96.2% purity, batch number: 2,015,602) was supplied by Spring Autumn Biological Engineering, Co., Ltd, Nanjing, China. Allopurinol, the positive control used in the experiments, was obtained from Xinyi Pharmaceutical Ltd, Shanghai, China. Protodioscin and allopurinol were suspended in 0.4% carboxymethylcellulose sodium (CMC-Na) solution for the animal experiments. Fructose was purchased from Aldrich Chemical Company (St Louis, MO, USA), Inc. Kits for blood glucose, blood urea nitrogen (BUN), creatinine (Cr), total cholesterol (TC), triglyceride (TG), UA and protein concentration were purchased from Nanjing Jiancheng Institute, Nanjing, China. Enzyme-linked immunosorbent assay kits for tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), leptin and insulin were obtained from Cusabio Biotech Co., Ltd, Wuhan, China. Antibodies of c-Jun N-terminal kinases (JNK), phosphorylated JNK (p-JNK), extracellular signal-regulated kinase (ERK), phosphorylated ERK (p-ERK), protein-38 mitogen activated protein kinases (p38 MAPK) and phosphorylated p38 (p-p38 MAPK) were purchased from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA. Antibodies against nuclear transcription factor-κB P65 (NF-κBP65), phosphorylated NF-κBP65 (p-NF-κBP65), inhibitor of NF-κB-α (IκBα) and phosphorylated IκBα (p-IκBα) were purchased from Signalway Antibody Co., Ltd, College Park, MD, USA. Horseradish peroxidase-conjugated antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was purchased from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA. Whole cell lysis assay kits and nuclei isolation lysis assay kits were purchased from Keyge Biotech Co., Ltd, Nanjing, China.

Animals

Adult male ICR mice (18–22 g) were purchased from Jiangsu University Laboratory Animal Center and kept at a maintained environment (23 ± 2 °C, humidity of 50% ± 10%, and 12 h light/dark cycle) with access to normal laboratory food and water. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, and were approved by the Ethics Committee of China Pharmaceutical University.

Experimental design and treatment protocol

After habituation for 7 days, the animals were randomly divided into either the control ($n = 10$) or experimental ($n = 40$) group. Mice in the control group received drinking water and standard chow, while the experimental group received 30% (w/v) fructose in drinking water and standard chow for 12 weeks (Yang et al., 2015). After 6 weeks, mice receiving high-dose fructose were divided into four subgroups: fructose group (treated with CMC-Na in a matched volume), allopurinol (Allo) group (administered 5 mg/kg allopurinol hydrochloride), protodioscin-5 (Pdio-5) group (administered 5 mg/kg protodioscin) and protodioscin-10 (Pdio-10) group (administered 10 mg/kg protodioscin). Dose of protodioscin was selected according to other reports and the clinical adult dose of DR (Wang et al., 2010). According to the pharmacopoeia of China, the dose of DR for human is 30 g/day. Equivalently, the calculated dose of DR based on respective body surface areas for rats is 2.6 g/kg/day. The average content of protodioscin in DR is 0.183% (Liu et al., 2006a), and so the dose of protodioscin for rats is 4.76 mg /kg/day. Therefore, we chose 5 mg/kg/day as low dose, and 10 mg/kg/day as high dose in this study. All drugs were administered orally once daily between 9:00 and 11:00 a.m., continuously for 6 weeks.

Glucose tolerance

Glucose tolerance was estimated by an oral glucose tolerance test (OGTT) at the end of the treatment. After 12 h of fasting, mice were administered 2 g/kg glucose orally and blood samples were collected from the caudal vein at 0, 30, 60, 90 and 120 min after glucose administration to measure the blood glucose levels. The results were expressed as an integrated area under glucose concentration time curve (AUC).

Urine, blood and tissue processing

Three days after the OGTT, urine samples were collected from mice, who were housed in individual metabolic cages for 24 h, for analysis of urine volume and biochemical parameters. The blood was collected via the abdominal aorta and centrifuged (3000 g) for 10 min to obtain serum for TC, TG, insulin, leptin, Cr, BUN and UA analysis. After being weighed, the right kidneys were collected for histology examination and the left kidneys were stored in liquid nitrogen for western blot analyses and biochemical estimations.

Histological examination of the mice kidneys

The kidneys from the mice were fixed in 10% neutral formalin for 24 h at room temperature, dehydrated through a graded alcohol series, embedded in paraffin, and cut into 4 μm-thick sections. The sections were stained with hematoxylin-eosin and the related indicators were examined under light microscope. Histological assessment was examined by two experienced morphologists, who were blinded to the origin of the slides. The surface area

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