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Curcumin ameliorates diabetic nephropathy by inhibiting the activation of the SphK1-S1P signaling pathway

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ABSTRACT

Curcumin, a major polyphenol from the golden spice *Curcuma longa* commonly known as turmeric, has been recently discovered to have renoprotective effects on diabetic nephropathy (DN). However, the mechanisms underlying these effects remain unclear. We previously demonstrated that the sphingosine kinase 1-sphingosine 1-phosphate (SphK1-S1P) signaling pathway plays a pivotal role in the pathogenesis of DN. This study aims to investigate whether the renoprotective effects of curcumin on DN are associated with its inhibitory effects on the SphK1-S1P signaling pathway. Our results demonstrated that the expression and activity of SphK1 and the production of S1P were significantly down-regulated by curcumin in diabetic rat kidneys and glomerular mesangial cells (GMCs) exposed to high glucose (HG). Simultaneously, SphK1-S1P-mediated fibronectin (FN) and transforming growth factor-beta 1 (TGF-β1) overproduction were inhibited. In addition, curcumin dose dependently reduced SphK1 expression and activity in GMCs transfected with $SphK^{WT}$ and significantly suppressed the increase in SphK1-mediated FN levels. Furthermore, curcumin inhibited the DNA-binding activity of activator protein 1 (AP-1), and c-Jun small interference RNA (c-Jun-siRNA) reversed the HG-induced up-regulation of SphK1. These findings suggested that down-regulation of the SphK1-S1P pathway is probably a novel mechanism by which curcumin improves the progression of DN. Inhibiting AP-1 activation is one of the therapeutic targets of curcumin to modulate the SphK1-S1P signaling pathway, thereby preventing diabetic renal fibrosis.

1. Introduction

Diabetic nephropathy (DN), one of the major microvascular complications of diabetes, has become the main cause of end-stage renal disease requiring dialysis (Molitch et al., 2004; Yang et al., 2010). Renal fibrosis is the main pathological feature of DN. DN is characterized by early glomerular hypertrophy, hyper-filtration, and accumulation of extracellular matrix (ECM) components such as fibronectin (FN). The disease is later stimulated by transforming growth factor-beta 1 (TGF-β1), triggering the thickening of glomerular and tubular basement membranes, which ultimately progress to glomerulosclerosis and renal fibrosis (Schena and Gesualdo, 2005). Multiple mechanisms contribute to the development and outcome of DN, such as oxidative stress, lipid disorders, renal hemodynamic changes, increased non-enzymatic glycosylation of proteins, and the activation of the polyol pathway and mitogen-activated protein kinase signaling pathway (Dronavalli et al., 2008; Evans et al., 2002).

Recently, the sphingosine kinase 1-sphingosine 1-phosphate (SphK1-S1P) signaling pathway has gained considerable attention because of its potential involvement in the progression of DN (Awad et al., 2011; Geoffroy et al., 2004). The formation of S1P is catalyzed by SphK1 (Ogretmen and Hannun, 2004). Hyperglycemia, advanced glycation end products (AGE), and oxidative stress activate SphK1 and thus increase the intracellular level of S1P (Geoffroy et al., 2004; Wang et al., 2005). S1P is one of the sphingolipid metabolites that function as both extracellular and intracellular signaling mediators in the regulation of diverse biological processes, such as cell proliferation, differentiation, migration,

Abbreviations: AGE, Advanced glycation end product; AP-1, Activator protein 1; BUN, Blood urea nitrogen; Cr, Serum creatinine; Cur, Curcumin; DN, Diabetic nephropathy; ECM, Extracellular matrix; FBG, Fasting blood glucose; FN, Fibronectin; GBM, Glomerular basement membrane; GMCs, Glomerular mesangial cells; HG, High glucose; KW/BW, Kidney weight-to-body weight ratio; LC-MS/MS, Liquid chromatography tandem-mass spectrometry; NG, Normal glucose; PAS, Periodic acid-Schiff; SD, Sprague Dawley; siRNA, Small interference RNA; S1P, Sphingosine 1-phosphate; SphK, Sphingosine kinase; SphK1-S1P, Sphingosine kinase 1-sphingosine 1-phosphate; STZ, Streptozocin; TGF-β1, Transforming growth factor-beta 1; UP, Albuminuria; 5C, SphK1 inhibitor 5C.

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adhesion molecule expression, angiogenesis, and vascular lesion resulting in various chronic vascular diseases including DN (Spiegel and Milstien, 2003; Takabe et al., 2008). As an intracellular second messenger, S1P activates TGF-β, mimics TGF-β-induced cellular response, and ultimately accelerates the renal fibrosis process (Xin et al., 2004). We previously demonstrated that the activation of the SphK1-S1P pathway involved in the elevated expression of FN in glomerular mesangial cells (GMCs) under diabetic conditions plays a pivotal role in the pathogenesis of DN (Lan et al., 2011a, 2010b; Liu et al., 2012).

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], the active constituent of the spice turmeric, is a multi-target natural compound that regulates multiple pathways, intracellular components, and key enzymes (Gupta et al., 2011; Zhou et al., 2011). With an outstanding safety profile, curcumin exhibits extensive pharmacological activities including anti-oxidant, anti-inflammatory, anti-proliferative, hypoglycemic, and immunomodulatory effects (Chainani-Wu, 2003; Nishiyama et al., 2005; Strimpakos and Sharma, 2008). Many of these contributed to ameliorating the development of DN. Recent studies have shown that curcumin improves renal function in streptozocin (STZ)-induced diabetic rats (Sharma et al., 2006; Tikoo et al., 2008). However, the mechanisms underlying the renoprotective effects of curcumin in DN remain unclear.

In short, the activation of SphK1-S1P signaling pathway induced by hyperglycemia and oxidative stress play important roles in renal fibrosis and DN. Curcumin possesses anti-oxidant and anti-inflammation properties. Therefore, the involvement of the renoprotective function of curcumin in the SphK1-S1P signaling pathway and in preventing diabetic renal fibrosis needs investigation. In this study, we explored the effects of curcumin on the SphK1-S1P signaling pathway and on expression of FN and TGF-β1 in the kidneys of type 1 diabetic rats induced by STZ and GMCs exposed to high glucose (HG). Both our *in vitro* and *in vivo* observations confirm that the down-regulation of the SphK1-S1P pathway may partially account for the role of curcumin in ameliorating the pathogenesis of diabetic fibrosis. The present study provides a novel mechanism underlying the renoprotective effects of curcumin and offers a new therapeutic strategy for the prevention and treatment of DN.

2. Materials and methods

2.1. Chemicals and reagents

Curcumin used for cell experiments, STZ, ATP, and α-tubulin antibody were procured from Sigma–Aldrich Corporation (St. Louis, MO, USA). Curcumin used for animal treatment (purity ⩾ 95%, HPLC) was purchased from Nanjing Zelang Pharmaceutical Technology Co., Ltd. (Nanjing, China). SphK1 inhibitor 5C (5C) was obtained from Cayman Chemical Company (Michigan, USA). S1P and C17-S1P were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco Invitrogen Corporation (Carlsbad, CA, USA). Newborn calf serum was obtained from PAA Cell Culture Company (Pasching, Austria). A nuclear extraction kit was obtained from Active Motif (California, USA). SphK1 rabbit polyclonal antibody was purchased from Abcam (Cambridge, UK). Antibodies against TGF-β1, c-Jun, and Flag were supplied by Cell Signaling Technology, Inc. (Danvers, MA, USA). FN mouse monoclonal antibody and specific c-Jun-siRNA were acquired from Santa Cruz Biotechnology Co. (Santa Cruz, CA, USA). Horseradish peroxidase (HRP)-conjugated secondary antibodies were acquired from Promega Corporation (Madison, USA). Polyvinylidene difluoride (PVDF) membranes were purchased from Immobilon®-PSQ (Millipore, CA, USA). TRIzol™, Lipofectamine™ 2000 Reagent, Lipofectamine® LTX & Plus Reagent, and Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) were purchased from Invitrogen Molecular Probes, Inc. (Eugene, OR, USA). SYBR Premix Ex Taq II was obtained from TaKaRa Biotechnology Co., Ltd. (Dalian, China). An enhanced chemiluminescence substrate for the detection of HRP and a LightShift® Chemiluminescent electrophoretic mobility shift assay (EMSA) kit were obtained from Thermo Fisher Scientific, Inc. (Rockford, USA). Biotin-labeled AP-1 consensus oligonucleotide was acquired from Sangon Biotech Co., Ltd. (Shanghai, China).

2.2. Animal treatment

The entire animal experiments were carried out under specific pathogen-free conditions in the Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China), in accordance with the China Animal Welfare Legislation and were approved by the Ethics Committee on the Care and Use of Laboratory Animals in Sun Yat-sen University. Male Sprague-Dawley (SD) rats were purchased from the Laboratory Animal Center of Sun Yat-sen University and housed with free access to regular food and water. After 1 week of acclimatization, experimental diabetes was induced by a single intraperitoneal injection of STZ (60 mg/kg) dissolved in ice-cold sodium citrate buffer (0.01 M, pH 4.5). Age-matched control rats received only sodium citrate buffer. At 72 h after the injection of STZ or citrate buffer, blood samples were collected via the tail vain, and fasting blood glucose (FBG) levels were measured using a one-touch ultra blood glucose meter (Johnson & Johnson Co.). Rats with FBG levels >16.7 mmol/L were considered diabetic. Diabetic animals were randomly divided into diabetic ($n = 8$) and curcumin-treated groups ($n = 8$). In the curcumin treatment group, the rats were administered with curcumin by gavage at a dose of 150 mg/kg. Curcumin was suspended in 1% sodium carboxymethyl cellulose (30 mg/ml) and was administrated in a constant volume of 5 ml/kg body weight. The same volume of vehicle (1% sodium carboxymethyl cellulose) was given to the rats in both the normal control and diabetic groups. Animal received once a day curcumin or the vehicle between 9:00 am and 10:00 am for 12 weeks.

2.3. Biochemical analysis

At the termination of the experiment, FBG levels were determined and the rats were weighed and housed in metabolic cages for 24 h to collect urine. After anesthesia, blood was collected from the abdominal aorta, and the serum was separated and stored at −20 °C until analysis. Blood urea nitrogen (BUN), serum creatinine (Cr), and 24 h albuminuria (UP 24 h) were analyzed by the Department of Pathology at the First Affiliated Hospital, Sun Yat-sen University. After the rats were euthanized, kidney samples were rapidly dissected, weighed, fixed in 4% paraformaldehyde solution or snap-frozen in liquid nitrogen, and then stored at −80 °C until analysis. Renal hypertrophy was assessed using the kidney-to-body weight ratio at the time of sacrifice.

2.4. Morphological studies

Renal tissues were fixed in 4% paraformaldehyde overnight and then embedded in paraffin. Sections (4 μm thick) were prepared and stained with periodic acid-Schiff (PAS). The degree of glomerulosclerosis and expansion of the mesangial matrix in the glomeruli in the sections were examined by light microscopy. Glomerular cell number was determined by counting the number of hematoxylin-stained nuclei. Glomerular hypertrophy was calculated from the cross-sectional area of the glomerular tuft. The cross section yielding the maximum diameter of the glomerulus was photographed and converted into a digital image by an examiner