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Original article

Curcumin ameliorates epithelial-to-mesenchymal transition of podocytes in vivo and in vitro via regulating caveolin-1

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ABSTRACT

Aims: Epithelial-mesenchymal transition (EMT) is recognized to play a key role in diabetic nephropathy (DN). Curcumin, the main active component of turmeric extracted from the roots of the *Curcuma longa* plant, has been reported for its anti-fibrotic effects in kidney fibrosis. The purpose of our study was to investigate the effects of curcumin in reversing epithelial-to-mesenchymal transition (EMT) of podocytes in vivo and in vitro.
Materials/methods: In vivo streptozotocin (STZ)-induced diabetic rats received vehicle or curcumin, and podocytes were treated with high glucose (HG) in the presence or absence of curcumin in vitro. And we investigated the effect of curcumin on HG-induced phosphorylation of cav-1 on the stability cav-1 and β-catenin using immunoprecipitation and fluorescence microscopy analysis.
Results: Curcumin treatment dramatically ameliorated metabolic parameters, renal function, morphological parameters in diabetic rats. We found that HG treatment led to significant down-regulation of p-cadherin and synaptopodin, as well as remarkable up-regulation of α-SMA and FSP-1 in vivo and in vitro. Furthermore, curcumin inhibited HG-induced caveolin-1 (cav-1) Tyr^{14} phosphorylation associating with the suppression of stabilization of cav-1 and β-catenin.
Conclusions: In summary, these findings suggest that curcumin prevents EMT of podocytes, proteinuria, and kidney injury in DN by suppressing the phosphorylation of cav-1, and increasing stabilization of cav-1 and β-catenin.

1. Introduction

Diabetic nephropathy (DN), one of the major detrimental long-term complications of diabetes mellitus (DM), has become the main cause of end-stage renal disease requiring dialysis [1]. Recent studies indicate that podocyte injury is a common trigger leading to the disruption of the filtration barrier and protein leakage, and ultimately result in glomerulosclerosis [2]. It is widely recognized that podocytes are able to undergo epithelial-to-mesenchymal transition (EMT) after chronic injury, a process that is believed to play a critical role in causing podocytes dysfunction that ultimately leads to a defective glomerular filtration [3,4]. However, the intracellular mediators that govern podocyte EMT remain unclear.

Caveolin-1 (cav-1) is the main constituent molecule of caveolae, which are omega-shaped plasma membrane invaginations enriched in cholesterol and sphingolipids [5]. Caveolae are thought to be signalling platforms regulating the activation of several signalling pathways. Cav-1 Tyr^{14} phosphorylation may function to facilitate cav-1 interaction with other proteins in a stimulus-specific fashion [6]. Cav-1 can be precipitated with E-cadherin and interacts with β-catenin in zebrafish [7]. Thus, cav-1 might have a more general role in regulating cell junctions, cav-1 recruits β-catenin to a Triton X-100-insoluble protein complex, presumably at the plasma membrane, thereby precluding Tcf/Lef-dependent transcription [8–12]. It is generally reported that the E-cadherin/catenin complex is functionally linked to actin filaments [13,14] and that this interaction is critical in junction regulation. β-catenin, a central structural component of this adhesion complex, also acts as a transcriptional co-activator in the Wnt/β-catenin signalling pathway, a pathway used to hastened EMT formation of podocytes [15]. The importance of linking the alteration of cav-1 and β-catenin complex expression may be a new way to understand the EMT of DN.

Curcumin is a natural polyphenolic compound derived from the root of *Curcuma longa* that regulates multiple pathways [16]. A large body of evidence from in vitro and in vivo studies of both animals and human have shown that curcumin has anti-fibrotic

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effect in kidney fibrosis [17–19]. Recent studies have shown that curcumin improves renal function in streptozotocin (STZ) -induced diabetic rats [20–24]. However, the mechanisms underlying the renoprotective effects of curcumin in DN have not been established.

Curcumin has been reported to modulate cav-1 and β-catenin signalling pathway as well [25–27]. Cav-1 is reduced by Chol:MβCD, and this down-regulation could be recovered by curcumin [28]. It has been reported that curcumin activates PKD1, resulting in changes in β-catenin signalling by inhibiting nuclear β-catenin transcription activity and enhancing the levels of membrane β-catenin in prostate cancer cells [29]. In the present study, we assessed the effects of curcumin on HG-induced EMT of podocytes in vivo and in vitro, and we hypothesized that curcumin may inhibit renal fibrosis as assessed by EMT through modulating the expression of cav-1 and the crosstalk between cav-1 and β-catenin signalling pathway. The present study provides a novel mechanism to understand the renoprotective effects of curcumin and offers a new therapeutic strategy for the treatment of DN.

2. Materials and methods

2.1. Experimental animals

All animal studies were carried out in accordance with the guidelines of the Animal Ethics Committee of The Shandong University. Male Wistar rats (8 weeks of age) weighing 250–300 g were obtained from Animal Centre of Shandong University and allowed 7 days for environmental and trainer-handling acclimation. The rats were fasted overnight before intraperitoneal injection of 60 mg/kg STZ (sigma, USA) in citrate buffer (pH, 4.5), while the control animals received 20 mM citrate buffer solution. Diabetes was confirmed by random blood-glucose levels higher than 16.7 mmol/L for 3 continuous days. The rats were randomly divided into three groups with eight rats in each group:

- non-diabetic control rats (Con group);
- STZ-induced diabetic rats (DM group);
- STZ-induced diabetic rats that treated with curcumin (sigma, USA) 100 mg/kg/day body weight [30,31] diluted in vehicle olive oil (DM+Cur group).

Curcumin was administered by gavage between 9:00 am and 10:00 am for 12 weeks. The same volume of vehicle (100 μL/100 g body weight olive oil) was given to the rats in both the normal control and diabetic groups. All rats were sacrificed at 12 weeks after the induction of diabetes for analysis of renal tissue. Rats were housed in a temperature-controlled room and were given free access to water and food during the study period.

2.2. Biochemical analysis

At the termination of the experiment, rats were weighed and fasting blood-glucose (FBG) levels were measured and housed in metabolic cages for 24 h to collect urine. The rats were given euthanasia by intraperitoneal injection of pentobarbital (50 mg/kg), blood was collected from left ventricle. Blood urea nitrogen (BUN), serum creatinine (Scr), and 24 h albuminuria (UP 24 h) were analysed by the Department of Pathology at the Second Hospital of Shandong University. Creatinine clearance (Ccr) was calculated in individual rats as follows: Creatinine clearance = urine creatinine × urine volume/plasma creatinine × time [32].

2.3. Histopathological analysis

To assess the glomerular sclerotic injury, renal tissues were fixed in 4% paraformaldehyde overnight and then embedded in paraffin. Four micrometer-thick sections were prepared and stained with periodic acid-Schiff (PAS) for histological evaluation, and also stained with Masson to demonstrate fibrosis in kidney tissues. Expansion of the mesangial matrix and glomerulosclerosis in the sections were assessed semi-quantitatively by light microscopy. The cross-section yielding the maximum diameter of the glomerulus was photographed and converted into a digital image by an examiner blinded to the tissue source using a light microscope equipped with a camera (NIKON ECLIPSE 90i, LH-M100CB-1, Japan). Glomerular tuft areas and mesangial areas were measured using image analysis software Image Pro-Plus (Media Cybernetics, Bethesda, MD). Fifty glomeruli were chosen at random from three slides in each animal and the average was used for analysis.

2.4. Electron microscopy

Renal cortex samples were cut into 1 mm^3 pieces on ice, immediately fixed in 2.5% glutaraldehyde. Glutaraldehyde fixed renal tissues were postfixed with 1% OsO_4 for 90 min and dehydrated with graded ethanol, and then embedded with resin (Epon 812) and polymerised at 72 °C for 48 h. Ultrathin sections were stained with lead acetate and uranyl, and were examined by electron microscopy. The morphologic assessment was performed independently by two blinded investigators.

2.5. Immunohistochemical staining

The localizations of α-SMA (abcam, USA), FSP-1 (abcam, USA), P-cadherin (Santa Cruz Biotechnology, USA), synaptopodin (Santa Cruz Biotechnology, USA), β-catenin (Cell Signaling, USA) and caveolin-1 (Cell Signaling, USA) in kidney tissues were measured by immunohistochemistry in prepared kidney sections. Immunohistochemical staining sections of the kidney samples were processed using a standard immunostaining protocol. After deparaffinization, hydration, and routine blockage of endogenous peroxidase, sections were pretreated with microwave for 20 min in Tris-EDTA (pH 9.0) for antigen retrieval, and then the sections were incubated overnight at 4 °C with primary antibody, and secondary antibody. After diaminobenzidine reaction, the slides were counterstained with hematoxylin. Imaging of all immunohistochemical sections was performed using a Leica microscope electronic imager. The expression of α-SMA, FSP-1, P-cadherin, synaptopodin, β-catenin and caveolin-1 was quantified by Image Pro-Plus v 6.0 software (Media Cybernetics, Inc) to analyze the integral optical density. All measurements were performed in a blinded fashion.

2.6. Cell culture and treatment

Conditionally immortalized mouse podocyte cell line (mpc5), kindly provided by Dr. Peter Mundel (Mount Sinai School of Medicine, New York), was cultured at 33 °C in RPMI 1640 medium (HyClone, USA) containing 10% fetal calf serum (Gibco, USA) and recombinant interferon-γ (Invitrogen, Carlsbad, California). After differentiated at 37 °C for 10–14 days without interferon-γ, podocytes were used for the proposed experiments. Podocytes were treated with low glucose (5.5 mM) as control group and HG (30 mM) without or with curcumin. Curcumin was dissolved in DMSO, and a similar volume of DMSO was added to controls. Mannitol (30 mM) was used as an osmolarity control.