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# Polydatin ameliorates experimental diabetes-induced fibronectin through inhibiting the activation of NF-κB signaling pathway in rat glomerular mesangial cells

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ABSTRACT

A number of studies have recently demonstrated the involvement of nuclear factor-kappa B (NF-κB) activation and the subsequent coordinated inflammatory responses in the pathogenesis of diabetic nephropathy (DN). Polydatin has been shown to have the ability of anti-adhesive inflammation. However, the possible protective and beneficial effects of polydatin on DN via suppressing inflammatory damage and extracellular matrix (ECM) accumulation are not fully elucidated. We found that the polydatin could inhibit the induction and activity of NF-κB, and meanwhile ameliorating ECM accumulation in streptozotocin-diabetic rats. We aimed to investigate the effect of polydatin on fibronectin (FN) protein expression, and to elucidate its potential mechanism involving the NF-κB inflammatory signaling pathway in rat glomerular mesangial cells (GMCs) cultured under high glucose. The results revealed that polydatin significantly suppressed high glucose-induced FN production, inhibited NF-κB nuclear translocation, reduced the DNA-binding activity of NF-κB, as well as decreased the protein expression of ICAM-1 and TGF-β in GMCs. These findings suggested that polydatin significantly represses high glucose-induced FN expression in rat GMCs, which may be closely related to its inhibition of the NF-κB signaling pathway. Hence, we elucidated the potential mechanisms of the anti-inflammatory effects and ECM accumulation alleviation of polydatin in GMCs of DN in vitro.



## 1. Introduction

Diabetic nephropathy (DN) is a morbid microvascular complication associated with diabetes, and is the most common cause of end-stage renal disease. In DN, the accumulation of extracellular matrix (ECM) components in the glomerular mesangium and tubulointerstitium causes early glomerular hypertrophy, as well as later glomerulosclerosis and tubulointerstitial fibrosis (Mauer et al., 1984; Adler, 1994). Glomerular mesangial cells (GMCs) play important roles in the physiological and pathological processes of kidneys. GMC has been postulated to be a key contributor to glomerulosclerotic lesions in diabetic patients (Young et al., 1995). GMC proliferation and hypertrophy, ECM accumulation, as well as consequent renal fibrosis have been recognized as major pathogenic events in the progression of renal failure in DN (Schena and Gesualdo, 2005; Ichinose et al., 2007; Raptis and Viberti, 2001; Tack et al., 2002; Chow et al., 2004). As one of the important ingredients of ECM, fibronectin (FN) is often used as an index to evaluate the extent of matrix accumulation. Inhibiting FN production is regarded as an effective strategy to ameliorate DN.

However, the pathogenesis of DN has not been fully elucidated. DN has been recently believed as a kind of chronic inflammation (Chow et al., 2004; Mora and Navarro, 2005; Navarro and Mora, 2005; Nelson et al., 2005; Saraheimo et al., 2003). An increasing number of studies have demonstrated that the activation of nuclear factor-kappa B (NF-κB) and the subsequent coordinated expression of gene products may play important roles in the pathogenesis of DN. In diabetes, activated NF-κB translocates into the nucleus and triggers the expression of target genes, including intercellular adhesionmolecule-1 (ICAM-1) and transforming growth factor-beta 1 (TGF-β1). Consequently, persistent and enhanced inflammation as well as excessive FN production is induced.

The root and rhizome of *Polygonum cuspidatum* have long been commonly used in traditional Chinese herbal medicine as an analgesic, antipyretic, diuretic, and expectorant. The major active component of *P. cuspidatum* is polydatin, also known as piceid, is a glucoside of resveratrol whose glucoside group bonded in the C-3

*Abbreviations:* NF-κB, nuclear factor-kappa B; DN, diabetic nephropathy; ECM, extracellular matrix; FN, fibronectin; GMCs, glomerular mesangial cells; ICAM-1, intercellular adhesionmolecule-1; TGF-β1, transforming growth factor-beta; STZ, Streptozocin; PDTC, pyrrolidinedithiocarbamate.

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position substitutes a hydroxyl group. Polydatin is more resistant to enzymatic oxidation than resveratrol and is soluble in water. Unlike resveratrol, which passively penetrates cells, polydatin enters cells via an active mechanism using glucose carriers (Fabris et al., 2008; Mikulski and Molski, 2010). These properties endow polydatin molecules with a greater bioavailability than resveratrol. Polydatin could obviously ameliorate inflammatory damage-induced intercellular adhesion and reduce the expression of adhesion molecules (Wang et al., 2002; Shu et al., 2004). These pharmacological activities of polydatin convey its influence on the field of cardio-cerebrovascular diseases.

Polydatin has been shown to ameliorate inflammatory damage-induced adhesion. However, the underlying mechanisms of this protective effect on suppressing inflammatory damage leading to excessive FN production as well as ECM accumulation, and later, glomerulosclerosis, are not fully elucidated in GMCs in DN. We firstly explored the expression of NF-κB and FN in kidney tissues of Streptozocin (STZ)-induced diabetic rats treated with polydatin. Furthermore, to reveal the effects of polydatin on FN production and the activation of NF-κB signaling pathways in high glucose-induced rat GMCs model in vitro, we used the NF-κB specific inhibitor ammonium pyrrolidinedithiocarbamate (PDTC) as a positive control. We finally elucidated the protective effects and potential mechanisms of polydatin in GMCs of DN to a certain extent, providing further mechanistic insight into the nephroprotective action of polydatin.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Streptozocin (STZ) was produced by Sigma–Aldrich Corporation (St. Louis, MO, USA). Polydatin used for animal (purity = 95.1%, HPLC) treatment was purchased from Shanxi Scidoor Hi-tech Biology Co., Ltd. (Batch No.: 20050220; Xi'An, China). Polydatin used for cell experiments, ammonium PDTC, and dimethyl sulfoxide were obtained from Sigma–Aldrich (St. Louis, MO, USA). Trypsin was from Hyclone Thermo Scientific (Rockford, USA). Penicillin/streptomycin solution and Dulbecco's modified Eagle's medium (DMEM) were from Gibco Invitrogen Corporation (Carlsbad, CA, USA). Newborn calf serum was from PAA Cell Culture Company (Pasching, Austria). A nuclear extraction kit was from Active Motif (CA, USA). An enhanced chemiluminescence (ECL) substrate for the detection of horseradish peroxidase (HRP) was obtained from Thermo Scientific (Rockford, USA). Antibodies against NF-κB p65, IκB-α, ICAM-1, and FN were supplied by Santa Cruz Biotechnology Co. (Santa Cruz, CA, USA). TGF-β rabbit monoclonal antibody was from Cell Signaling Technology, Inc. (Danvers, MA, USA). HRP-conjugated secondary antibodies were from Promega Corporation (Madison, USA). α-Tubulin antibody was obtained from Sigma–Aldrich (St. Louis, MO, USA). A polyvinylidene difluoride (PVDF) membrane was purchased from immobilon®-PSQ (Millipore, CA, USA). Alexa Fluor® 488 Goat Anti-Mouse IgG (H + L) was from Invitrogen Molecular Probes, Inc. (Eugene, OR, USA). A LightShift® Chemiluminescent electrophoretic mobility shift assay (EMSA) kit was obtained from Pierce Thermo Scientific (Rockford, USA).

### 2.2. Animal experiment

Male Sprague–Dawley (SD) rats ($n$ = 30, bodyweight: 200 ± 10 g) from Laboratory Animal Center, Sun Yat-sen University, Guangzhou, China. All animal procedures conformed to the China Animal Welfare Legislation and were reviewed and approved by the Sun Yat-sen University Committee on Ethics in the Care and Use of Laboratory Animals. (Animal quality certificate No.: 0005201). All animals were housed under standard conditions with free access to regular food and water. After fed with regular diet for 1 week, they were randomly assigned to an Streptozocin-induced diabetic group ($n$ = 22), which were given a single tail-vein injection of freshly prepared STZ (60 mg/kg), and a normal control group ($n$ = 8), which were injected with equal volume of citrate buffer alone. Diabetic rats were confirmed by the levels of fasting blood glucose measurement $\geqslant$16.7 mmol/l after 72 h injection, which were randomized (8/group) to receive polydatin (150 mg/kg, i.g. daily) or orally given equal volumes of physiological saline. Control rats were also fed equal volumes of physiological saline. Treatment was continued for 12 weeks, at which time rats were sacrificed. The rats were housed in individual metabolic cages for collection of urine on the day before the end of the experiment. Blood sample was collected by drainage from the retroorbital venous plexus. Serum was obtained by centrifuge at 3000$g$ for 15 min and stored at −80 °C. Meanwhile, kidney samples were rapidly excised, weighed and frozen in liquid nitrogen and stored at −80 °C or fixed in 10% neutral-buffered formalin.

### 2.3. Biochemical analysis and morphological studies

Blood glucose was determined by glucose oxidase method. Blood urea nitrogen and serum creatinine were measured by oxidase and phosphoglycerol oxidase dynamical enzyme method, respectively. Urine protein was detected by sulfosalicylic acid–sodium sulfate turbidity method (available kits from Beijing Chemclin Biotech Co., Ltd., Beijing, China). For morphometric studies, the kidneys were fixed in 10% neutral buffered formalin and subsequently embedded in paraffin. The 4-μm sections of paraffin-embedded tissues were stained with periodic acid-Schiff (PAS). The cross section yielding the maximum diameter of the glomerulus was photographed and converted into a digital image by an examiner, blinded to the source of the tissue, using light microscopy equipped with camera (Olympus BX-50; Olympus Optical, Tokyo, Japan). Glomerular tuft areas were measured with image analysis software Image Pro. Plus (Media Cybernetics, Inc., Bethesda, MD, USA). Twenty glomeruli were chosen at random form 3 slides in each animal as previous report (Peng et al., 2008).

### 2.4. Immunohistochemistry

Sections (4-μm thick) of kidney were processed using a standard immunostaining protocol. After deparaffinization, hydration and blockage of endogenous peroxidase routinely, sections were pretreated by microwave for 20 min in 10 mmol/L sodium citrate buffer (pH 6.0) for antigen retrieval, followed by incubation sequentially with blocking agent, mouse anti-NFκB p65 antibody (1:1000, Santa Cruz, CA, USA) and secondary antibody (1:50). Slides were counterstained with hematoxylin after 3 min of diaminobenzidine reaction, and cover slipped using Vectashield (Vector Labs, Burlingame, CA, USA), then photographed and converted to a digital image using light microscopy equipped with camera. Negative control was carried out by omitting the primary antibody.

Positive staining (dark brown) for NFκB p65 in each glomerulus was quantified by two investigators in a blinded manner at a magnification of 400× using image analysis software Image Pro. Plus, and expressed as the ratio of the mean of normal SD rats. Twenty glomeruli were chosen at random form 3 slides in each animal as previous report (Peng et al., 2008) (total 160 glomeruli for each group).

### 2.5. Cell culture

Rat GMCs were separated from the glomeruli of SD rats by our research group and identified with a specific assay as previously