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Polydatin attenuates AGEs-induced upregulation of fibronectin and ICAM-1 in rat glomerular mesangial cells and *db/db* diabetic mice kidneys by inhibiting the activation of the SphK1-S1P signaling pathway

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ABSTRACT

We previously demonstrated that activation of sphingosine kinase 1 (SphK1)- sphingosine 1- phosphate (S1P) signaling pathway by high glucose (HG) plays a pivotal role in increasing the expression of fibronectin (FN), an important fibrotic component, by promoting the DNA-binding activity of transcription factor activator protein 1 (AP-1) in glomerular mesangial cells (GMCs) under diabetic conditions. As a multi-target anti-oxidative drug, polydatin (PD) has been shown to have renoprotective effects on experimental diabetes. However, whether PD could resist diabetic nephropathy (DN) by regulating SphK1-S1P signaling pathway needs further investigation. Here, we found that PD significantly reversed the upregulated FN and ICAM-1 expression in GMCs exposed to AGEs. Simultaneously, PD dose-dependently inhibited SphK1 levels at the protein expression and kinase activity and attenuated S1P production under AGEs treatment conditions. In addition, PD reduced SphK activity in GMCs transfected with wild-type $SphK^{WT}$ plasmid and significantly suppressed SphK1-mediated increase of FN and ICAM-1 levels under normal conditions. Furthermore, we found that the AGEs-induced upregulation of phosphorylation of c-Jun at Ser63 and Ser73 and c-Fos at Ser32, DNA-binding activity and transcriptional activity of AP-1 were blocked by PD. In comparison with *db/db* model group, PD treatment suppressed SphK1 levels (mRNA, protein expression, and activity) and S1P production, reversed the upregulation of FN, ICAM-1, c-Jun, and c-Fos in the kidney tissues of diabetic mice, and finally ameliorated renal injury in *db/db* mice. These findings suggested that the downregulation of SphK1-S1P signaling pathway is probably a novel mechanism by which PD suppressed AGEs-induced FN and ICAM-1 expression and improved renal dysfunction of diabetic models.

Abbreviations: AGEs, advanced glycation-end products; AP-1, activator protein 1; BUN, blood urea nitrogen; C17−S1P, C17-D-erythro-sphingosine 1-phosphate; C17-sph, C17-D-erythro-sphingosine; COX-2, cyclooxygenase-2; Cr, serum creatinine; DMEM, dulbecco's modified eagle's medium; DN, diabetic nephropathy; ECM, extracellular matrix; FBG, fasting blood glucose; FN, fibronectin; GBM, glomerular basement membrane; GMCs, glomerular mesangial cells; HG, high glucose; ICAM-1, intercellular adhesion molecule-1; KW/BW, kidney weight to body weight ratio; LC-MS/MS, liquid chromatography tandem-mass spectrometry; MMI, mesangial matrix index; NG, normal glucose; NF-κB, nuclear factor-kappa B; PAS, periodic acid-Schiff; PD, polydatin; RBG, random blood glucose; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; siRNA, small interfering RNA; SphK, sphingosine kinase; TGF-β1, transforming growth factor-beta 1; UP, urine protein; 5C, SphK1 inhibitor 5C.

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1. Introduction

Diabetic nephropathy (DN), one of the most severe microvascular complications of diabetes mellitus, is also the main cause of end-stage renal disease (Park, 2014; Schena, 2005). The main pathological characteristics of DN are glomerulosclerosis and tubulointerstitial fibrosis (Kanwar et al., 2011). Under diabetic conditions, proliferation and hypertrophy of glomerular mesangial cells (GMCs), accumulation of extracellular matrix components, such as fibronectin (FN), and excessive secretion of inflammation factors, such as intercellular adhesion molecule-1 (ICAM-1), trigger the expansion of mesangial area and thickening of glomerular basement membrane (GBM), leading to renal fibrosis that eventually develops into DN (Ichinose et al., 2007; Kim et al., 1995). The mechanisms responsible for DN are complicated, and multiple factors, including augmented non-enzymatic glycosylation of proteins, oxidative stress, and activation of the inflammation pathway, contribute to the pathological progress of DN (Dronavalli et al., 2008; Evans et al., 2002; Schena, 2005). However, the exact pathogenesis of DN has not been fully elucidated.

Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid that regulates diverse biological processes, such as cell proliferation, differentiation, and migration (Ogretmen and Hannun, 2004). S1P can be secreted out of the cell as a ligand for its specific receptors or stay intracellularly as a second messenger (Spiegel and Milstien, 2003). Sphingosine kinase 1 (SphK1) is the key metabolic enzyme that catalyzes the synthesis of S1P via sphingosine-phosphorylation (Pitson, 2011). Recently, the SphK1-S1P signaling pathway has gained increased attention for its role in the pathogenesis of renal fibrosis and DN (Geoffroy et al., 2005; Xin et al., 2004). Hyperglycemia, advanced glycation-end products (AGEs), and oxidative stress can activate the SphK1-S1P signaling pathway and result in GMCs proliferation and accumulation of fibrotic components (Geoffroy et al., 2004; Takuwa et al., 2010; Wang, 2005). We previously demonstrated that activation of the SphK1-S1P signaling pathway is a pivotal regulator of elevated FN expression and that the function of the SphK1-S1P signaling pathway is closely correlated with AP-1 activation (Huang et al., 2014; Lan et al., 2011). Therefore, inhibiting SphK1-S1P activation is extremely important to delay the progression of DN.

Polydatin (PD), also known as piceid, can be isolated from the roots of *Polygonum cuspidatum Sieb. et Zucc* (Fig. 1) (Xing et al., 2009). PD has been shown to improve heart function, ameliorate renal injury and resist Alzheimer's disease through multiple pharmacological activities, like anti-oxidation and anti-inflammation (Chen et al., 2013; Jiang et al., 2013; Rivière et al., 2009). We have previously confirmed that PD obviously ameliorates renal function of experimental diabetic rats and successfully suppresses FN accumulation in GMCs exposed to high glucose by inhibiting activation of the nuclear factor-kappa B (NF-κB) signaling pathway, which eventually results in inhibited renal fibrosis and DN resistance (Xie et al., 2012). Given the marked anti-oxidative and anti-inflammatory effects of PD, whether the anti-DN effect of PD is related to its regulation of oxidative stress-induced activation of SphK1-S1P signaling pathway requires further investigation.

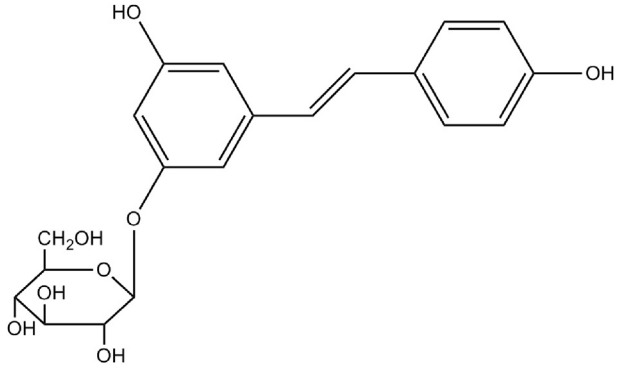

Fig. 1. The chemical structure of PD.

The current study explored the levels of SphK1-S1P, FN, and ICAM-1 in GMCs exposed to AGEs, and then confirmed the observations in *db/db* diabetic mice. We demonstrated that PD suppresses the expression of FN and ICAM-1 in AGEs-treated GMCs through inhibition of the SphK1-S1P pathway to postpone the pathological progression of DN.

2. Materials and methods

2.1. Reagents and antibodies

D-glucose was purchased from Amresco (Solon, OH, USA). Bovine serum albumin (BSA, Fraction V) was obtained from Mbchem (Shanghai, China). PD used for cell treatment was purchased from Kechuang (Beijing, China), PD used in animal experiment was obtained from Zelang (purity > 98.0%, HPLC; Nanjing, China). SphK1 inhibitor 5C (5C) was obtained from Cayman Chemical Company (Ann Arbor, Michigan, USA). C17-D-erythro-Sphingosine 1-Phosphate (C17–S1P), S1P, and C17-D-erythro-sphingosine (C17-sph) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). ATP was purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Polyvinylidene difluoride (PVDF) membranes were purchased from Immobilon®-PSQ (Millipore, MA, USA). Nuclear Extract Kit was purchased from Active Motif (Carlsbad, CA, USA).Antibodies against FN, ICAM, OctA(FLAG®) (Santa Cruz Biotechnology, Santa Cruz, CA, USA); α-tubulin (Sigma); SphK1 used for WB,c-Fos, c-Jun, phospho-c-Fos (Ser32), phospho-c-Jun (Ser63), phospho-c-Jun (Ser73) (Cell Signaling Technology, Boston, MA, USA); SphK1 used for immunohistochemistry (Boster Immunoleader, Wuhan, Hubei, China); horseradish peroxidase-conjugated secondary antibodies (Promega, Madison, WI, USA); and goat anti-rabbit IgG labeled with Alexa Fluor 594 (Molecular Probes, Eugene, OR, USA) mouse advanced glycation end products (AGEs) ELISA kits (Cusabio Biotech, Wilmington, DE, USA) were purchased from commercial sources.

2.2. Preparation and characterization of AGEs

AGEs-BSA (AGEs) was produced using D-glucose and fatty acid free BSA using previously described protocol (Huang et al., 2013a). Briefly, 0.8 g of BSA (40 mg/mL) was incubated with 1.8 g of D-glucose in 20 mL of PBS (0.2 M, pH 7.4) under sterile conditions at 37 °C for 90 days without light. All preparations of AGEs were dialyzed in 10 mM of PBS (pH 7.4) for 96 h to remove the free glucose and passed over detoxigel columns (Detoxi-Gel™ Endotoxin Gel; Thermo Fisher Scientific, Rockford, IL, USA) to remove endotoxin. Endotoxin levels in preparations were further determined via limulus amebocyte lysate testing (Houshiji, Xiamen, China), and were found to be less than 0.01 EU/mL. Estimation of glycation by spectrofluorometry (PerkinElmer, Waltham, MA, USA) with excitation wavelength of 370 nm and emission wavelength of 440 nm revealed approximately 50-fold increase in characteristic fluorescence for AGEs as compared with control.

2.3. Cell culture

Rat primary GMCs were obtained from Sprague Dawley rat kidney cortex fragments using standard protocols as described