Molecular and Cellular Endocrinology 399 (2015) 178–189

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Polydatin promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs-induced upregulation of fibronetin and transforming growth factor-β1 in rat glomerular messangial cells

Kaipeng Huang [a,b], Cheng Chen [b], Jie Hao [b], Junying Huang [b], Shaogui Wang [b], Peiqing Liu [b], Heqing Huang [b,*]

[a] Department of Pharmacy, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China
[b] Laboratory of Pharmacology & Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form 10 August 2014
Accepted 29 August 2014
Available online 2 September 2014

Keywords:
Diabetic nephropathy
Advanced glycation-end products
Oxidative stress
Sirt1
Polydatin
Nrf2-ARE pathway

ABSTRACT

Sirt1 and nuclear factor-E2 related factor 2 (Nrf2)-anti-oxidant response element (ARE) anti-oxidative pathway play important regulatory roles in the pathological progression of diabetic nephropathy (DN) induced by advanced glycation-end products (AGEs). Polydatin (PD), a glucoside of resveratrol, has been shown to possess strong anti-oxidative bioactivity. Our previous study demonstrated that PD markedly resists the progression of diabetic renal fibrosis and thus, inhibits the development of DN. Whereas, whether PD could resist DN through regulating Sirt1 and consequently promoting Nrf2-ARE pathway needs further investigation. Here, we found that concomitant with decreasing RAGE (the specific receptor for AGEs) expression, PD significantly reversed the downregulation of Sirt1 in terms of protein expression and deacetylase activity and attenuated FN and TGF-β1 expression in GMCs exposed to AGEs. Under AGEs-treatment condition, PD could decrease Keap1 expression and promote the nuclear content, ARE-binding ability, and transcriptional activity of Nrf2. In addition, PD increased the protein levels of heme oxygenase 1 (HO-1) and superoxide dismutase 1 (SOD1), two target genes of Nrf2. The activation of Nrf2-ARE pathway by PD eventually led to the quenching of ROS overproduction sharply boosted by AGEs. Depletion of Sirt1 blocked Nrf2-ARE pathway activation and reversed FN and TGF-β1 downregulation induced by PD in GMCs challenged with AGEs. Along with reducing HO-1 and SOD1 expression, silencing of Nrf2 increased FN and TGF-β1 levels. PD treatment elevated Sirt1 and Nrf2 levels in the kidney tissues of diabetic rats, then improved the anti-oxidative capacity and renal dysfunction of diabetic models, and finally reversed the upregulation of FN and TGF-β1. Taken together, the resistance of PD on upregulated FN and TGF-β1 induced by AGEs via oxidative stress in GMCs is closely associated with its activation of Sirt1-Nrf2-ARE pathway.

1. Introduction

Diabetic nephropathy (DN), one of the common chronic microvascular complications of diabetes, is the leading cause of end-stage renal failure (Kanwar et al., 2008, 2011). The main pathological changes of DN are characterized by glomerular mesangial cells (GMCs) proliferation and hypertrophy and extracellular matrix (ECM) accumulation, which ultimately result in the occurrence of renal fibrosis including glomerulosclerosis (Kanwar et al., 2008, 2011; Mariappan, 2012). As intrinsic cells of glomerulus, GMCs play vital roles in maintaining the organizational structure and physiological function of kidney by generating ECM, secreting cytokines, phagocytosing and clearing foreign bodies, and regulating intraglomerular capillary flow (Abboud, 2012; Gruden et al., 2005). The massive accumulation of fibronectin (FN), an important component of ECM, and excessive secretion of transforming growth factor-β1 (TGF-β1) in the glomerular mesangium are critical indicators of diabetic renal fibrosis that eventually leads to DN (Gruden et al., 2005; Kanwar et al., 2008, 2011).

The pathological mechanisms of DN are ascribed to the integrated effects of multiple factors, such as activated rennin-angiotensin

Abbreviations: AGEs, advanced glycation-end products; ARE, anti-oxidant response element; BSA, bovine serum albumin; DN, diabetic nephropathy; ECM, extracellular matrix; EMSA, electrophoretic mobility shift assay; FN, fibronectin; GMCs, glomerular mesangial cells; HG, high glucose; HO-1, heme oxygenase 1; Keap1, kelch like ECH-associated protein 1; SOD, superoxide dismutase; MDA, malondialdehyde; NAD^+, nicotinamide adenosine dinucleotide$^+$; Nrf2, nuclear factor E2-related factor 2; OSS, oxidative stress; PD, polydatin; RAGE, the specific receptor for AGEs; ROS, reactive oxygen species; STZ, streptozocin; TGF-β1, transforming growth factor-β1.

* Corresponding author. Laboratory of Pharmacology & Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, 132 East Circle at University Town, Guangzhou 510006, China. Tel.: +86 020 39943028; fax: +86 020 39943110.
E-mail address: huangheq@mail.sysu.edu.cn (H. Huang).

http://dx.doi.org/10.1016/j.mce.2014.08.014
0303-7207/

system in local renal tissue, advanced glycation-end products (AGEs), polyol pathway dysfunction, abnormal cytokine network, oxidative stress (OSS), and susceptible gene background (Gruden et al., 2005; Kanwar et al., 2008, 2011; Tripathi and Yadav, 2013). Numerous studies have demonstrated that the increased AGEs formed by glycometabolic disorder in the kidney tissues under diabetic conditions are the major causative pathological mechanism for DN (Forbes and Cooper, 2012; Kanwar et al., 2011). By binding to its specific receptor-RAGE, AGEs can boost the excessive production of reactive oxygen species (ROS) in renal tissues and cells, which not only directly decrease the expression of manganese superoxide dismutase (SOD) in rat GMCs, but also induce the generation of profibrotic factors by activating mitogen-activated protein kinase, nuclear factor-kappa B, and protein kinase C to promote renal cell proliferation or hypertrophy and increase FN and TGF-β1 expression, ultimately initiating and participating in the development of diabetic renal fibrosis (Kashihara et al., 2010; Lu et al., 2013; Singh et al., 2011; Yamagishi and Matsui, 2010). Thus, suppressing the overproduction of FN and TGF-β1 induced by AGEs through OSS is of remarkable significance for DN treatment.

Sirt1, a key member of the mammalian sirtuin family, is a nicotinamide adenosine dinucleotide^{+} (NAD^{+})-dependent protein deacetylase (Houtkooper et al., 2012; Rahman and Islam, 2011). Using many histones and non-histone proteins as substrates, Sirt1 possesses remarkable anti-oxidative capacity (Radak et al., 2013; Wang et al., 2011; Webster et al., 2012) and participates in various cell biology processes including gene transcription silencing, cell growth cycle, energy metabolism, insulin secretion, angiogenesis, and cellular senescence (Houtkooper et al., 2012; Rahman and Islam, 2011). Recent studies found that Sirt1 protein expression is downregulated in the kidney tissues of diabetic *db/db* mice and GMCs exposed to high glucose (HG) (Kim et al., 2013). Small molecular activators targeting Sirt1 have potential applications in the pretreatment of DN (Kim et al., 2013; Li et al., 2010; Wu et al., 2012). Our previous study has demonstrated that AGEs reduce the protein expression and deacetylase activity of Sirt1 and therefore, increase FN and TGF-β1 levels in a dose- and time-dependent manner in rat GMCs; and activation of Sirt1 can inhibit the upregulation of FN and TGF-β1 through promoting nuclear factor-E2 related factor 2 (Nrf2)-anti-oxidant response element (ARE) anti-oxidative pathway (Huang et al., 2013), suggesting a crucial regulatory function of Sirt1 and Nrf2 in the pathological progression of AGEs-induced DN.

Polydatin (PD), a resveratrol glucoside with a 3, 4', 5-trihydroxystilben-3-β-D-mono-D-glucoside molecular structure, is an active component isolated from the roots of *Polygonum cuspidatum* Sieb. et Zucc (Fig. 1) (Fabris et al., 2008). PD has multiple pharmacological activities, especially strong anti-oxidative effects (Chen et al., 2013; Jiang et al., 2013; Su et al., 2013), and has been shown to protect heart function (Jiang et al., 2013) and ameliorate Alzheimer's disease (Riviere et al., 2009). We've previously confirmed that PD ameliorates the renal dysfunction of experimental diabetic rats and alleviates FN accumulation by inhibiting the nuclear factor-kappa B signaling pathway in GMCs and therefore, preventing the development of diabetic renal fibrosis (Xie et al., 2012). Given the remarkable anti-oxidative effects of PD, we then determined whether the resistance of PD on DN is correlated with regulating Sirt1 and promoting Nrf2-ARE pathway, which deserve further investigation.

Based on the aforementioned, this study was designed to observe whether PD could regulate Sirt1 function and inhibit FN and TGF-β1 expression through promoting the activation of Nrf2-ARE pathway in GMCs challenged with AGEs. Here, we showed that concomitant with reversing AGEs-induced downregulation of Sirt1, PD significantly increased the nuclear content, ARE-binding ability, and transcriptional activity of Nrf2, and then upregulated the expression of Nrf2 target genes-heme oxygenase 1 (HO-1) and superoxide dismutase 1 (SOD1). Importantly, the PD-induced activation of Sirt1-Nrf2-ARE anti-oxidative pathway eventually quenched ROS generation sharply boosted by AGEs, suppressed FN and TGF-β1 expression and thereby, slowed down the progression of DN.

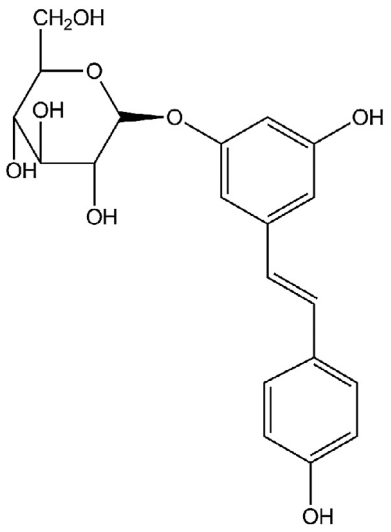

Fig. 1. The chemical structure of PD.

2. Material 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 cells treatment) was purchased from Weiye (Beijing, China), PD used in animal experiment was obtained from Zelang (purity > 98.0%, HPLC; Nanjing, China). Streptozocin (STZ) were supplied by Sigma (St Louis, MO, USA). Antibodies against FN, Sirt1, RAGE, and Nrf2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); TGF-β1 (Cell Signaling Technology, Boston, MA, USA); α-tubulin and Histone 1.4 (Sigma); HO-1 (Epitomics, Burlingame, CA, USA); Keap1 and SOD1 (Proteintech Group, Chicago, IL, USA); horseradish peroxidase-conjugated secondary antibodies (Promega, Madison, WI, USA); and goat anti-rabbit IgG labeled with Alexa Fluor® 594 (Molecular Probes, Eugene, OR, 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., 2013). 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 condition at 37 °C for 90 days. 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.