Biomedicine & Pharmacotherapy 98 (2018) 18–22

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Protocatechuic acid ameliorates high glucose-induced extracellular matrix accumulation in diabetic nephropathy

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ARTICLE INFO

Keywords:
Protocatechuic acid (PCA)
Diabetic nephropathy
Mesangial cells (MCs)
High glucose

ABSTRACT

Protocatechuic acid (PCA), a phenolic compound of anthocyanins, was reported to possess various pharmacologic properties, including anti-oxidant, anti-inflammatory, anti-apoptosis, anti-diabetic and anti-tumor activities. However, the role of PCA in diabetic nephropathy remains elusive. The present study was conducted to evaluate the effects of PCA on extracellular matrix (ECM) accumulation in high glucose (HG)-induced human mesangial cells (MCs) and explore the possible mechanism. Our results demonstrated that PCA obviously inhibited HG-induced proliferation of MCs in a dose-dependent manner. In addition, PCA effectively reduced the protein expression levels of type IV collagen, laminin and fibronectin induced by HG, as well as decreased the levels of ROS and MDA in HG-stimulated MCs. Mechanistic studies showed that PCA efficiently down-regulated the phosphorylation level of p38 MAPK in HG-stimulated MCs. Taken together, our present study demonstrated that PCA protects MCs against HG damage might via inhibition of the p38 MAPK signaling pathway. Thus, PCA might be a beneficial agent for the prevention and treatment of diabetic nephropathy.

1. Introduction

Diabetic nephropathy is a common microvascular complication in diabetic patients. It is characterized by excessive deposition of extracellular matrix (ECM) components, often resulting in the development of glomerulosclerosis and tubulointerstitial fibrosis [1]. Despite improved prognosis in the past years, the pathogenesis of diabetic nephropathy has not been fully elucidated. Diabetic nephropathy is caused by a variety of factors, such as hyperglycemia, hypertension, oxidative stress, chemokines, and inflammation [2,3]. Previous investigations have demonstrated that hyperglycaemia plays a critical role in the pathogenesis of diabetic nephropathy [4–6]. High glucose can promote the proliferation and ECM synthesis in glomerular mesangial cells (MCs) [7]. Therefore, developing effective approaches to suppress HG-induced MC proliferation and ECM accumulation is of significant importance for prevention of diabetic nephropathy.

Protocatechuic acid (PCA) is a phenolic compound of anthocyanins. Emerging evidence has demonstrated that PCA possesses various pharmacologic properties, including antioxidant, anti-inflammatory, anti-apoptosis, antidiabetic and anti-tumor activities [8–10]. One study reported that PCA could stimulate the insulin signaling pathway in human adipocytes through increasing GLUT4 translocation and glucose uptake [11]. However, the role of PCA in diabetic nephropathy remains elusive. The present study was conducted to evaluate the effects of PCA on ECM accumulation in HG-induced human MCs and explore the possible mechanism. Our results demonstrated that PCA suppressed HG-induced cell proliferation and ECM expression in MCs might through inhibition of the p38MAPK signaling pathway.

2. Materials and methods

2.1. Cell culture

Normal human MC line was purchased from the Shanghai Academy of Life Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS, Gibco BRL, USA), 100 mg/ml streptomycin, and 100 U/ml penicillin (Invitrogen, Carlsbad, CA) at 37 °C under an atmosphere of 5% CO_2. The cells were used at confluence between the 5th and 7th passages.

2.2. LDH release assay

MCs were seeded at a density of 1×10^4 cells/well in 96-well culture plates for 24 h, and then treated with various concentrations of PCA (≥98%, Sigma; 5, 10 and 20 μM) for 30 min before incubation

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https://doi.org/10.1016/j.biopha.2017.12.032
Received 22 October 2017; Received in revised form 29 November 2017; Accepted 7 December 2017

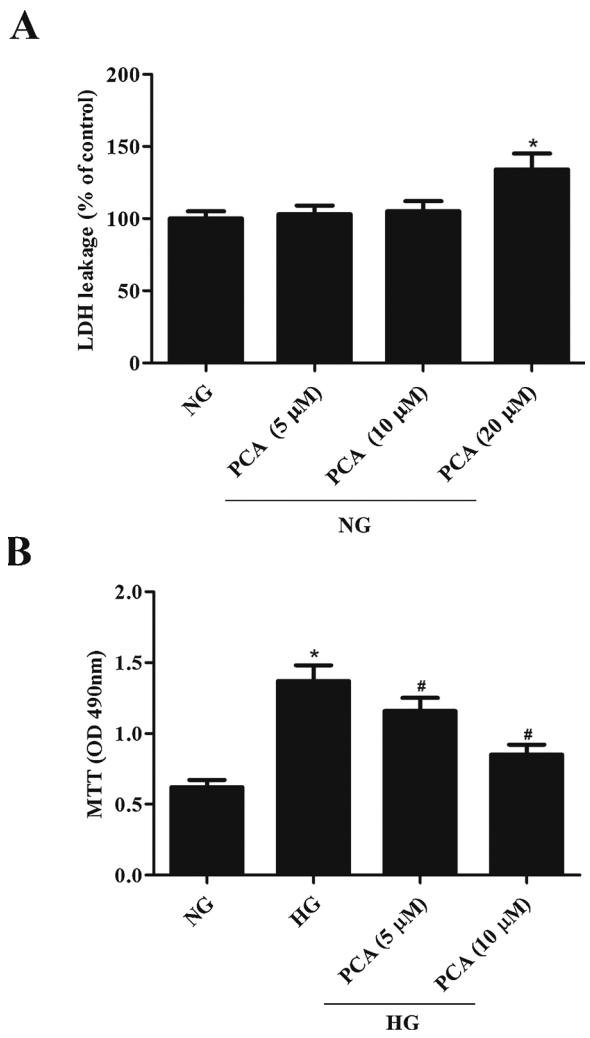

Fig. 1. PCA inhibits cell proliferation induced by HG in MCs. A, MCs were pretreated with and without PCA (5, 10 and 20 μM) for 30 min before incubation with 5.5 mM normal glucose (NG) for 24 h. Cell cytotoxicity was evaluated by the LDH assay. B, MCs were pretreated with and without PCA (5 and 10 μM) for 30 min before incubation with 30 mM high glucose (HG) for 24 h. Cell proliferation was detected using the MTT assay. Data is expressed as mean ± SD. Experiments were performed in triplicate. $^{*}P < 0.05$ compared with the NG group, $\#P < 0.05$ compared with the HG group.

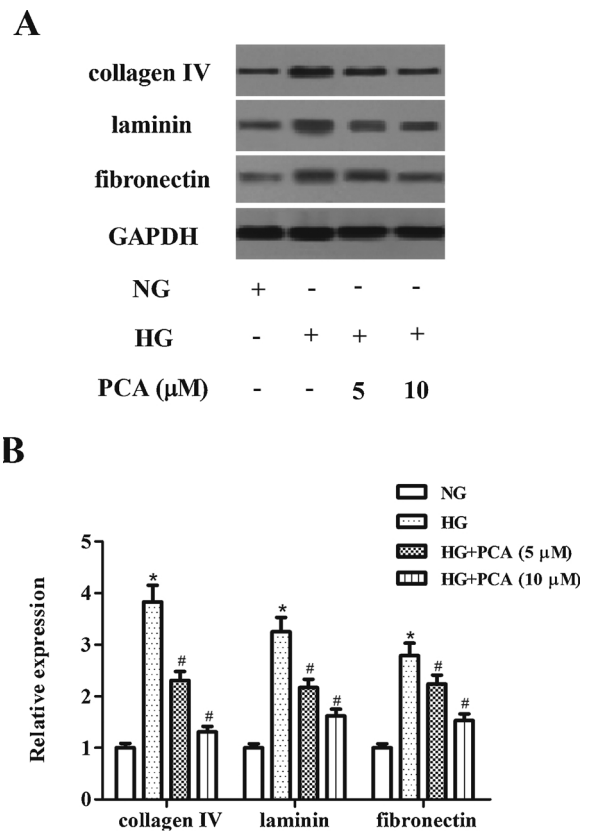

Fig. 2. PCA suppresses ECM expression induced by HG in MCs. A, MCs were pretreated with or without PCA (5 and 10 μM) for 30 min before incubation with 30 mM HG for 24 h. The protein expression levels of type IV collagen, laminin and fibronectin were detected with western blot. B, The relative protein expression levels of type IV collagen, laminin and fibronectin were quantified using Image-Pro Plus 6.0 software and normalized to GAPDH. Data is expressed as mean ± SD. Experiments were performed in triplicate. $^{*}P < 0.05$ compared with the NG group, $\#P < 0.05$ compared with the HG group.

with 5.5 mM normal glucose (NG) for 24 h. LDH release was examined by LDH cytotoxicity assay kit (Beyotime, Nantong, Jiangsu, China) in accordance with the manufacturer's instructions. Absorbance at 450 nm was measured using a microplate reader (Takara Biotechnology, Dalian, China). Cell death rate was calculated as follows: cell death rate = 100 × (experimental release − spontaneous release)/(maximum release − spontaneous release).

2.3. Cell proliferation assay

Cell proliferation was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). In brief, MCs were seeded at a density of 1×10^4 cells/well into 96-well plates. When cells reached confluence, cells were serum starvation for 24 h. Then, cells were incubated with 30 mM (high glucose [HG]) in the presence or absence of PCA (5 and 10 μM) for 24 h. Then, 20 μl of MTT (5 mg/ml; Sigma) was added to each well and incubation continued at 37 °C for 4 h, the supernatants were removed, and 150 μl of dimethylsulfoxide (DMSO; Sigma) was added to each well. The absorbance at 490 nm was evaluated with a microplate reader (Takara Biotechnology, Dalian, China). The cells incubated in control medium containing normal glucose concentration of 5.5 mM (NG group) were considered as control group.

2.4. Western blot analysis

Cells were harvested and lysed in RIPA buffer (Beyotime Biotechnology, Shanghai, China) containing 100 mM NaCl, 50 mM Tris-HCl pH 7.5, 1% Triton X-100, 1 mM EDTA, 10 mM β-glycerophosphate, 2 mM sodium vanadate and protease inhibitor. The cell lysate supernatants were harvested by centrifugation at 10,000 × *g* for 10 min at 4 °C. The concentration of protein was detected using a BCA™ Protein Assay Kit (Pierce, USA). Equal amounts of protein (40 μg of protein/lane) were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (Millipore, Bedford, MA, USA). Nonfat 5% milk was used to block for 1 h, and then the membranes were incubated with specific antibodies for type IV collagen (1:1500), laminin (1:2000), fibronectin (1:2500), NOX4 (1:3000), phospho-p38MAPK (1:2000), total-p38MAPK (1:1500) and GAPDH (1:2000) [Santa Cruz Biotechnology, Santa Cruz, CA, USA] overnight at 4 °C. Then, the membranes were washed with Tris-buffered saline including Tween-20 (0.1%) for three times and incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse immunoglobulin G (Santa Cruz Biotechnology) diluted 1:2000 in the blocking buffer for 1 h at room temperature. Finally, immune complexes were visualized using an enhanced chemiluminescence system (ECL, Pierce Company, USA).