European Journal of Pharmacology 824 (2018) 185–192

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Berberine attenuates podocytes injury caused by exosomes derived from high glucose-induced mesangial cells through TGFβ1-PI3K/AKT pathway

Ying-Ying Wang[a,b], Li-Qin Tang[b,*], Wei Wei[a,*]

[a] Institute of Clinical Pharmacology, Anhui Medical University, 81#Mei-shan Road, Hefei 230032, Anhui, People's Republic of China
[b] Department of Pharmacy, Anhui Provincial Hospital, Anhui Medical University, 17# Lu-jiang Road, Hefei 230001, Anhui, People's Republic of China

ARTICLE INFO

Keywords:
Diabetic nephropathy
Exosomes
Podocyte
Berberine
TGFβ1

ABSTRACT

Diabetic nephropathy is the most common microvascular complications of diabetes. Berberine is the main active ingredient of *Coptis chinensis* and previous studies have been showed that berberine could delay the progression of diabetic nephropathy by regulating related cytokines and signaling pathways. Glomerular mesangial cells and podocytes are two vital indigenous cells of kidney and interaction between these two cellular components via exosomes might affect function of glomerulus in diabetic nephropathy condition. On the basis of our previous studies, transwell systems were used to demonstrate that the exosomes released by glomerular mesangial cells induced by the high glucose were involved in podocytes injury. The current study demonstrates that berberine can reduce TGFβ1 in exosomes released by high glucose-induced glomerular mesangial cells. Berberine-treated high glucose-induced exosomes which are secreted by glomerular mesangial cells can protect damage of podocytes by reducing apoptosis and increasing adhesion. These results suggest that berberine could protect the function of podocytes through inhibiting the transfer of TGFβ1 from the glomerular mesangial cells to the podocytes, which is one of the potential mechanisms of protective effect of berberine on diabetic nephropathy.

1. Introduction

Diabetic nephropathy (DN) is one of the most common microvascular complications of diabetes end-stage renal failure (Wouters et al., 2015). The main clinical features of DN include incremental renal failure, proteinuria, increased serum and urine creatinine levels. Glomerular mesangial cells (GMCs) and podocytes are two types of resident cells in kidney, both of which involve in the process of DN.

Previous study has found that GMCs and podocytes interact in IgA nephropathy (Leung et al., 2015). TGFβ1 is a cytokine superfamily that affects a variety of cellular functions, including cell growth, differentiation, inflammation and apoptosis, and TGFβ1 has been proven that could cause glomerular sclerosis and DN. Elevated TGFβ1 is associated with loss of podocytes, which is an early feature of DN (Lee, 2012; Saitoh, 2015; Sedeek et al., 2013). Studies have shown that high glucose (HG) environment could induce TGFβ1 upregulation in renal tubular epithelial cells and activate extracellular matrix deposition in glomeruli by activating PI3K / AKT pathway (Wu et al., 2009).

Traditionally, it is believed that the crosstalk between cells is mainly achieved by direct contact and cytokine-receptor interaction. In the past decade, however, the discovery of exosomes expends our understanding about cell-cell interaction. Exosomes contain a variety of biological components, including different types of proteins, lipids, RNA and DNA. Upon being released from cells, exosomes could be uptaken by recipient cells and then exert their unique biological effects within recipient cells (Colombo et al., 2014). Previous study showed that HG could stimulate glomerular endothelial cells to secrete exosomes, which could activate GMCs. This interaction could consequently promotes renal interstitial fibrosis (Wu et al., 2016). Therefore in the present study, we firstly aimed at investigating whether exosomes derived from HG-induced GMCs involve in podocytes injury.

Berberine (BBR) is an isoquinoline alkaloid that isolated from *Coptidis rhizoma*. BBR exhibits multiple pharmacological activities, such as decreasing blood glucose and anti-oxidative stress and repressing inflammation. Therefore BBR is a potential therapeutic drug in DN treatment (Tang et al., 2014, 2016, 2013; Yang et al., 2014). However, it remains unclear whether BBR could inhibit renal fibrosis and protect podocytes dysfunction by affecting interaction between GMCs and podocytes.

In the current study, we firstly demonstrated that HG-induced GMCs could impair podocytes function via secreting exosomes. We then investigated whether BBR could attenuate the injury of podocytes that caused by exosomes derived from HG-induced GMCs. In addition, the underlying mechanism of the protective effect of BBR on podocytes

* Corresponding authors.
E-mail addresses: wangyingying601@163.com (Y.-Y. Wang), tulcyl@vip.sina.com (L.-Q. Tang), wwei@ahmu.edu.cn (W. Wei).

https://doi.org/10.1016/j.ejphar.2018.01.034
Received 28 December 2017; Received in revised form 19 January 2018; Accepted 23 January 2018

injury was also investigated. Taken together, our results show a novel mechanism that BBR could attenuates podocytes injury caused by exosomes derived from HG-induced GMCs through TGFβ1-PI3K/AKT signal pathway, which providing a potential target of DN treatment.

2. Materials and methods

2.1. Preparation of BBR

BBR was isolated and extracted from *Coptidis rhizoma* as described previously (Tang et al., 2013). The purity of BBR was 96.5% based on reversed-phase high-performance liquid chromatography (RP-HPLC, 600E pumps, 2996 PDA ultraviolet spectrophotometric detector, Waters Co., USA). The samples were analyzed on a C_{18} column (250 mm*4.6 mm, 5 μm) with acetonitrile-0.033 mol/l potassium dihydrogen phosphate solution (48:52) as the mobile phase; the flow rate was 1.0 ml/min, the detection wavelength was 263 nm, and the column temperature was 25 °C.

2.2. GMCs culture and transfection

Rat GMCs were separated from the glomerular of SD rats and identified with a specific assay (Tang et al., 2013). GMCs were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C under 5% CO2 atmosphere and subcultured using 0.25% trypsin every 3–4 days. At sub confluence, GMCs were incubated with serum-free DMEM for 24 h and divided into the following groups: normal glucose group (NG; 5.6 mmol/l glucose + 24.5 mmol/l mannitol), HG group (HG; 30 mmol/l glucose), HG plus BBR 50 μM group (BBR50μM + HG), HG plus BBR 100 μM group (BBR100μM + HG), HG plus TGFβ1 siRNA group (TGFβ1siRNA + HG), HG plus BBR 100 μM and TGFβ1 siRNA group (BBR 100μM + TGFβ1siRNA + HG). The BBR treatment concentrations were chosen based on cck-8 assays. After another 24 h of continuous culturing, the culture medium was collected for exosomes isolation according to the manufacture's protocol. A brief protocol can be found in ‘exosomes isolation, analysis and neutralization’ part. Podocytes were divided into six groups: podocytes without exosome treatment (Control group), podocytes incubated with NG-treated GMCs-derived exosomes (NG-GMCs-EXO group), podocytes incubated with HG-treated GMCs-derived exosomes (HG-GMCs-EXO group), podocytes incubated with HG plus BBR 50 μM-treated GMCs-derived exosomes (BBR50μM + HG-GMCs-EXO group), podocytes incubated with HG plus BBR 100 μM-treated GMCs-derived exosomes (BBR100μM + HG-GMCs-EXO group), podocytes incubated with HG plus TGFβ1 siRNA-treated GMCs-derived exosomes (TGFβ1siRNA + HG-GMCs-EXO group), podocytes incubated with HG plus TGFβ1 siRNA and BBR 100 μM-treated GMCs-derived exosomes (BBR 100μM + TGFβ1siRNA + HG -GMCs-EXO group).

For transfection study, GMCs were transfected with siRNA TGFβ1 for 24 h and treated with HG or BBR for another 24 h. The GMCs were then washed twice with PBS and replaced with fresh medium. After another 24 h of continuous culturing, the culture medium was collected for exosome isolation. A transfection kit ribo FECT™ CP (Ribobio, Guangzhou, China) was used. SiRNA TGFβ1 was also purchased from Ribobio and the transfected concentration was 50 nM.

2.3. Podocytes culture and intervention

Primary podocytes were purchased from All Cells (Shanghai, China) and cultured with a RPMI 1640 supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C under a 5% CO2 atmosphere. The 6–10 generations were used for our study and cells were collected for experiment after treatment with exosomes for 24 h. To make our data more reliable, three independent experiments were performed.

2.4. Co-culture of GMCs and podocytes

To clarify whether exosomes are involved in the process of podocytes injury caused by HG-induced GMCs, we co-cultured GMCs with podocytes in a Transwell-6 system with a 0.4-μm porous membrane (Corning, NY, USA) to prevent direct cell contact. The podocytes were planted in the lower wells and grown for an appropriate period of time. The GMCs were then planted in the upper wells. GW4869 (Sigma, California, USA), a specific neutral sphingomyelinase 2 inhibitor, also known as an inhibitor of exosomes release, was used at a concentration of 10 μM to reduce the release of exosomes from GMCs (Li et al., 2013). Before GMCs were co-cultured with podocytes, they were stimulated with GW4869 for 8 h.

2.5. Exosomes isolation, analysis and neutralization

Exosomes-free conditioned medium was collected at the indicated time-points after cell treatment. Exosomes were obtained through a series of centrifugation. In brief, the conditioned medium were sequentially centrifuged at 300 g for 10 min, 2000 g for 15 min, and 10,000 g for 30 min to remove cell debris and large vesicles, and then the cleared sample was further ultracentrifuged for 70 min at 100,000 g twice to pellet the exosomes (Tauro et al., 2012). The purified exosomes were resuspended in PBS and stored them at −80 °C subsequent studies.

The size distribution of exosomes were analyzed by nanosight (Malvern, UK). Protein markers, CD63, TSG101 and calnexin were determined by western blotting.

For up-take studies, purified exosomes were labelled with a PKH67 (green) kit (Sigma, California, USA) according to protocols. Briefly, exosomes diluted in PBS were added to 0.5 ml Diluent C. In parallel, 4 μl PKH67 dye was added to 0.5 ml Diluent C and incubated with the exosome solution for 4 min at room temperature. To bind excess dye, 2 ml 0.5% bovine serum albumin/PBS was added. The labelled exosomes were washed at 100,000 g for 70 min, and the exosome pellet was suspended with PBS and used for uptake experiments. After cells were fixed, they were visualized with a laser confocal scanning microscopy (LCSM) (Leica TCS-SP2, Wetzlar, Germany).

2.6. Transmission electron microscope

The exosomes were prepared and diluted in PBS, and then placed 20 μl suspension liquid onto formvar carbon-coated copper grids for 1 min at room temperature. The excess suspension liquid was removed by filter paper. Next, the exosomes were stained by 2% phosphotungstic acid at room temperature for 5 min. After that, grids were fixed with 2% glutaraldehyde for 5 min, followed by washing with PBS for three times. Images were captured under 80 kV with a transmission electron microscope (JEM-1230; Jeol Ltd., Tokyo, Japan).

2.7. CCK-8 assay

The effect of BBR on cell viability proliferation was determined using the CCK-8 assay. Cells were plated in triplicate at 2×10^5 per well and treated with BBR (12.5–150 μM) for 24 h. After treatment, 10 μl of CCK-8 solution was added to each well, and cells were continuously incubated for 1 h at 37 °C and 5% CO_2. The optical densities (ODs) of wells were examined at the test wavelength of 450 nm (each group had 5 wells).

2.8. Cell apoptosis assay

Following the experimental treatments , The analysis of cell apoptosis was performed using flow cytometry and the Annexin V-FITC/PI apoptosis detection kit (MultiSciences, Hangzhou, China). Briefly, the podocytes were plated in 6-well plates at a density of 1×10^5 cells each