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Resveratrol improves mitochondrial function in the remnant kidney from 5/6 nephrectomized rats

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ABSTRACT

Mitochondrial dysfunction is involved in the pathogenesis of chronic kidney disease (CKD). Resveratrol has been demonstrated to be beneficial for the recovery of kidney diseases. In this study, the 5/6 nephrectomized rat was used as a CKD model and the TGF-β1-exposed mouse mesangial cells were used as an *in vitro* model. Pathological examination showed that resveratrol treatment attenuated glomerular injury in the remnant kidney of 5/6 nephrectomized rat. Additionally, resveratrol improved mitochondrial function *in vivo* and *in vitro*, as evidenced by increasing mitochondrial membrane potential, increasing ATP, decreasing reactive oxygen species production and enhancing activities of complex I and III. Furthermore, the dysregulated expressions of electron transport chain proteins and fission/fusion proteins in the kidney of 5/6 nephrectomize rats and TGF-β1-exposed mesangial cells were restored by resveratrol. Finally, upregulated sirt1 and PGC-1α deacetylation were found after treatment with resveratrol *in vivo* and *in vitro*, which may contribute to the mitochondrial protective effects of resveratrol. The results demonstrate that resveratrol protects the mitochondria of kidney in 5/6 nephrectomized rats and TGF-β1 induced mesangial cells. The study provides new insights into the renoprotective mechanisms of resveratrol.

1. Introduction

Chronic kidney disease (CKD) is a major public health problem which affects approximate 10% of patients worldwide (Zhang and Rothenbacher, 2008). Glomerulosclerosis is a common pathological feature in CKD (Abboud, 2012). The most important profibrogenic mediator in CKD is TGF-β1 (Border and Noble, 1997; Lopez-Hernandez and Lopez-Novoa, 2012), which contributes to the development of glomerulosclerosis in CKD through promoting glomerular extracellular matrix (ECM) accumulation (Douthwaite et al., 1999). TGF-β1 facilitates the synthesis and inhibits the degradation of various ECM components in glomerular mesangial cells (Baricos et al., 1999; Poncelet and Schnaper, 1998). TGF-β1 has also been found to suppress the transcriptional activity of the genes associated to mitochondrial biogenesis and functions (Sohn et al., 2012). Mitochondria are important energy-producing organelles. The role of mitochondrial dysfunction has been widely studied in various diseases including kidney diseases since the first description of mitochondrial diseases (Luft et al., 1962). Martin-Hernandez and colleagues reported a high frequency of renal involvement in children with mitochondrial diseases (Martin-Hernandez et al., 2005). Gucer and colleagues reported two children with focal segmental glomerulosclerosis associated with mitochondrial cytopathy in podocytes (Gucer et al., 2005). In addition, impaired mitochondrial activity has been detected in CKD patients (Granata et al., 2009). These findings provide clinical evidence that abnormal mitochondria are involved in kidney diseases. Experimental studies also demonstrated that maintenance of mitochondrial function could protect renal function against kidney diseases. Moreover, mitochondrial dysfunction was found to be involved in the pathogenesis of epithelial-mesenchymal transition in renal proximal tubular epithelial cells (Yuan et al., 2012; Zhang et al., 2007). Several studies have proved that improving mitochondrial function was beneficial for the recovery of kidney diseases (Chen et al., 2013; Funk et al., 2010; Funk and Schnellmann, 2013). Therefore, mitochondria may be a potential target for CKD treatment.

Resveratrol is a polyphenolic phytoalexin produced by plants including grape, peanut, blueberry et al. It exerts various bioactivities such as cardioprotection, anti-inflammation, antioxidant, anti-cancer, anti-aging, and anti-diabetes (Carter et al., 2014; Liu et al., 2015a,b; Rege et al., 2014; Szkudelski and Szkudelska, 2014; Tao et al., 2015; Zordoky et al., 2015). *In vivo* and *in vitro* studies have demonstrated the potential of resveratrol in treating kidney diseases (Elbe et al., 2015;

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Huang et al., 2014; Pan et al., 2014; Zhang et al., 2014). It has been found that resveratrol promoted renal function *via* regulating nitric oxide pathway (Chander and Chopra, 2006) and Smad pathway (Huang et al., 2014). The mitochondrial protective effect of resveratrol has been shown in various tissues including kidney (Bosutti and Degens, 2015; Wang et al., 2015; Wang et al., 2014). The aim of this study was to further investigate the mechanisms of the renoprotective effects of resveratrol from the point of mitochondrial dysfunction in 5/6 nephrectomized rats and TGF-β1-callenged mouse mesangial cells.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats weighing 180–200 g were obtained from the Experiment Animal Center of Jinzhou Medical University (Jinzhou, China). The rats were housed in a temperature- and humidity-controlled environment with a 12/12 h light-dark cycle and maintained under a standard laboratory diet with water and libitum. All the animal experiments were approved by the Institutional Animal Care and Use Committee of Jinzhou Medical University.

2.2. Surgical procedure and drug administration

A total of 116 rats were divided into four groups as follows: sham (n = 18), sham + resveratrol (20 mg/kg) (sham + Res, n = 18), 5/6 nephrectomy (Nx, n = 40), and 5/6 nephrectomy + resveratrol (Nx + Res, n = 40). Rats were anesthetized with 10% chloral hydrate solution *via* intraperitoneal injection (Hung et al., 2014; Liu et al., 2015a; Yuksel et al., 2009). Rats underwent a 5/6 nephrectomy in which lower and upper thirds of the left kidney was ablated and the right kidney was removed. Rats in the two sham groups received sham operations. The majority of survival rats recovered well from the surgery. Rats received 20 mg/kg resveratrol (purity: > 98%, Guanyu Ltd. Co., Xi'an, China) or vehicle (10% dimethyl sulfoxide, DMSO) daily by gavage 1 week after the surgery for 4 weeks. Twenty-one rats in the Nx group and 18 rats in the Nx + resveratrol group died during the experiment. Six rats in each group were randomly selected for histological examinations, 6 were randomly selected for molecular biological examinations, and 6 were randomly selected for examinations of mitochondrial functions. The remaining rats were used for some preliminary experiments.

2.3. Kidney histopathology

Kidney tissues from the rats were fixed with 4% paraformaldehyde in 0.01 M PBS buffer overnight, embedded in paraffin, and then cut into 5-μm-thickness sections. After being dewaxed and gradient ethanol hydration, the sections were stained with periodic acid schiff (PAS) reagent (Baso Diagnostic Inc., Zhuhai, China) according to the manufacture's protocol and observed under an optic microscopy (DP73; Olympus, Tokyo, Japan).

2.4. Cell culture

Mouse mesangial cell line SV40 MES 13 (Type Culture Collection of Chinese Academy of Sciences, Shanghai, China) was cultured in dulbecco's modified eagle medium (DMEM) medium (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal calf serum (Hyclone, Logan, UT, USA). The cultures were maintained at 37 °C in a 5% CO_2 atmosphere. Mycoplasma contamination in the cells was tested using a commercial PCR Mycoplasma Test Kit (Huabio, Hangzhou, China) following the manufacture's instruction. The results were shown in the Supplementary Fig. S1 in Supplementary material in online version at DOI: http://dx.doi.org/10.1016/j.acthis.2017.04.002. The cells at passage 3 were used for the following experiments. After 12 h serum-starve, the cells were treated with 10 μM resveratrol (purity: 98%, Sigma-Aldrich, St Louis, MO, USA) and incubated with or without 2 ng/mL TGF-β (dissolved in PBS, Cloud-Clone Corp, Houston, TX, USA) for 24 h. The resveratrol was dissolved in 50% ethanol as an 80 mM storage solution and diluted into 10 μM with PBS.

2.5. Isolation of mitochondria

Remnant kidney cortexes which had been cut into pieces or mesangial cells were incubated in mitochondria isolation buffer (Beyotime Institute of Biotechnology, Haimen, China) supplemented with phenylmethanesulfonyl fluoride and homogenized and centrifuged at 1000*g* for 5 min at 4 °C. The supernatants were collected gently and centrifuged at 11,000*g* for 10 min at 4 °C. The pellet was collected as mitochondria. The protein concentration of mitochondrial fractions was measured using a Bicinchoninic Acid (BCA) protein assay kit (Beyotime Institute of Biotechnology, Haimen, China).

2.6. Mitochondrial membrane potential (MMP) measurement

The MMP (ΔYm) was measured using 5,-5′,-6, 6′-tetrachloro-1,-1-′,-3,-3-′-tetrethyl benzimidalyl carbocyanine iodide (JC-1, Invitrogen, Carlsbad, CA, USA). The isolated mitochondria or mesangial cells were incubated with JC-1 working solution at 37 °C for 20 min and washed twice in incubation buffer. The MMP of isolated mitochondria was determined using a Tube Luminometer (Lumat LB9507, Berthold, Pforzheim, Germany) with fluorescence intensity was set as red (excitation 525 nm/emission 590 nm) and green (excitation 488 nm/emission 525 nm) wavelengths. The MMP were calculated as the ratio of red to green fluorescence and normalized by the sham group. The MMP of mesangial cells were analyzed at 488 nm excitation and 530 nm emission using a flow cytometer (BD Biosciences, San Jose, CA, USA) and were expressed as the loss of MMP and normalized by the control group (Xie et al., 2016).

2.7. Mitochondrial and intracellular reactive oxygen species (ROS) assay

The formation of ROS in the mitochondria and cells was detected using DCFH-DA (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Isolated mitochondria and mesangial cells were incubated with DCFH-DA at 37 °C for 30 min. In the presence of ROS, DCFH reacted with ROS to form the fluorescent product dichlorofluorescein (DCF). The excess DCF-DA was washed off and the fluorescence of DCF was determined at 485 nm excitation and 525 nm emission using a flow cytometer (FACSCalibur, BD Biosciences, San Jose, CA, USA). Results were normalized by the sham or control group.

2.8. ATP content determination

ATP content in the kidney cortex and mesangial cells was measured using an ATP Determination Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The assay was based on the phosphorylation of creatine to generate phosphocreatine, which can be quantified by colorimetric (λ_{max} = 636 nm) method.

2.9. Activities of mitochondrial complexes I and III determination

The activities of complexes I and III were measured in the kidney and mesangial cells using Complexes I and III Activity Assay kits (Genmed, Shanghai, China) according to the manufacturer's instructions. Complex I activity was detected spectrophotometrically at 340 nm at 30 °C based on the rate of the oxidation of NADH, and complex III activity was monitored at 550 nm at room temperature based on the reduction of cytochrome c in the presence of reduced decylubiquinone using an ELISA reader (ELX-800, BioTek, VT, USA).