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Original Article

Protective effect of calcitriol on podocytes in spontaneously hypertensive rat

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Abstract

Background: Hypertension is a major global public health issue. Uncontrolled hypertension leads to organ damage, especially renal damage. Calcitriol is used to treat osteoporosis, promote bone formation, and increase bone mass. Previous studies have demonstrated that 1,25(OH)2D3, in addition to its classic role, also has multiple immune regulation and renoprotective functions and inhibits the activity of the renin-angiotensin-aldosterone system (RASS). The aim of the current study was to investigate the renoprotective effects of calcitriol in a spontaneously hypertensive rat (SHR) model.
Methods: A total of 18 SHRs and 8 age-matched normal Wistar rats were enrolled. SHRs were randomly divided into a hypertensive nephropathy group (H), a hypertensive nephropathy treated with calcitriol group (D) and a control group (NS). The rats were sacrificed after 16 weeks of treatment. The blood pressure (BP) of rats were measured one time every 4 weeks. The levels of serum albumin, serum creatinine, blood calcium, serum Vitamin D and 24-h urinary protein were measured after 16 weeks treatment. The protein level of WT1, nephrin and vitamin D receptor (VDR) was examined by Western blotting and immunohistochemical staining.
Results: There were no notable changes in blood pressure or serum creatinine in group H and D compared with group NS. The albumin, calcium and vitamin D serum levels in group H were significantly decreased compared with group NS and significantly increased in group D compared with group H. The level of 24-h urine protein significantly increased in group H compared with group NS and significantly decreased in group D compared with group H. The expression of VDR, WT1 and nephrin in the kidney were all significantly decreased in group H compared with group NS and significantly increased in group D compared with group H.
Conclusion: The present results indicated that there was injury of podocytes in hypertensive nephropathy, which can be ameliorated by calcitriol in SHR, but there was no significant anti-hypertensive effect. Vitamin D/VDR decreased proteinuria perhaps by increasing expression of nephrin and WT1 protein in podocyte of SHRs.

Keywords: Calcitriol; Hypertension; Hypertensive nephropathy; Podocyte

1. Introduction

Hypertension is a major global public health issue. Uncontrolled hypertension leads to organ damage, especially renal damage, that can finally lead to end-stage renal disease (ESRD).[1] Proteinuria is one of the sensitive indicators of early renal damage in patients with hypertension. Proteinuria is regarded as an important factor influencing the prognosis of hypertension; it is not only one of the characteristics of hypertensive renal impairment but is also an important risk factor for the progression of ESRD.[2] The current clinical treatment for hypertensive nephropathy mainly consists of the application of antihypertensive drugs, mainly angiotensin converting

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enzyme inhibitors and angiotensin receptor blockers. The main mechanism of antihypertensive drugs may be related to decreasing oxidative stress, correction of chronic hypoxia, and inhibition of advanced glycation end-product formation and of abnormal iron deposition.[3−6]

The incidence of chronic kidney disease (CKD) is increasing year by year and has become one of the major public health issues of global concern, suggesting that current treatments are not effective.[7] At the same time, emerging clinical and animal studies have demonstrated that active vitamin D and its analogues can attenuate glomerular damage and tubular interstitial fibrosis.[8] Treatment with 1,25-dihydroxy-vitamin D (1,25(OH)2D3 or calcitriol) or activated vitamin D analogues reduced albuminuria and prevented podocyte injury in a subtotal nephrectomy model,[9,10] puromycin-induced nephropathy,[11,12] adriamycin-induced nephropathy,[11,13] diabetic nephropathy,[3,14,15] immunoglobulin A nephropathy,[16,17] and in CKD patients.[18,19] However, the mechanism of the antiproteinuric effect of vitamin D remains unknown.

Previous studies have demonstrated that 1,25(OH)2D3, in addition to its classic role of regulating calcium and phosphorus metabolism to maintain normal function of the skeletal system, also has multiple immune regulation and renoprotective functions, inhibits the activity of the renin-angiotensin-aldosterone system (RASS), and helps regulate insulin secretion and the function of the nervous system.[19,20] The pleiotropic biological activities of 1,25(OH)2D3 are mediated by the vitamin D receptor (VDR), a member of the nuclear hormone receptor superfamily, including the membrane vitamin D receptor (mVDR) and nuclear receptor (nVDR). mVDR is mainly involved in the maintenance of calcium and phosphorus balance, and nVDR regulates the synthesis of protein by affecting gene expression. We mainly studied the expression of VDR in the glomerular podocytes.[20] VDR is expressed in numerous types of tissues and cells in the body, including cells not involved in calcium and phosphate metabolism. This is the basis for the broad biological function of VDR beyond the regulation of calcium and phosphorus.

The aforementioned research suggests that 1,25(OH)2D3 or activated vitamin D analogues have renoprotective functions. However, it is unclear if vitamin D modulates blood pressure (BP). Observational studies have found associations between vitamin D, increased BP and the risk of developing hypertension.[21,22] In contrast, recent data from randomized trials are mixed.[23] The present study aimed to investigate the protective effect of active vitamin D (calcitriol) on hypertensive nephropathy and to identify its protective mechanism. These data may be useful in developing a novel approach for the treatment of hypertensive nephropathy.

2. Methods

2.1. Reagents

Calcitriol was purchased from Sigma−Aldrich (Merck KGaA, Darmstadt, Germany). Primary antibodies used in this study were rabbit anti-nephrin (Abcam Ltd. Shanghai, China, ab136894), rabbit anti-VDR (Santa Cruz Biotechnology, Inc. sc1008), mouse anti-WT1 (Santa Cruz Biotechnology, Inc. sc393498), rabbit anti-GAPDH (Abcam Ltd. Hong Kong, China, ab9485). Anti-mouse secondary antibodies (115-035-003) and anti-rabbit secondary antibodies (111-035-003) were purchased from Jackson ImmunoResearch Laboratories Inc (West Grove, PA, USA).

2.2. Animals

A total of 18 adult male (purchased from Charles River) 10-week-old SHRs (SHR/NCrl, 121, RT1k), weighing 200 ± 10 g, and 8 adult male, 10-week-old Wistar rats (Crl:WI,102), weighing 200 ± 10 g, were enrolled. Rats were housed separately in cages with a 12-h dark/light cycle and 40%−70% relative humidity, at 18°C-22 °C temperature. Food and water were available ad libitum. All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.3. Animal treatment

Animals were randomly divided into 3 groups: a hypertensive nephropathy group (group H), a hypertensive nephropathy treated with calcitriol group (group D; intragastric administration of calcitriol 3 ng/100 g body weight per day) and a normal control group (group NS). The rats were sacrificed after 16 weeks of treatment. During the treatment period, rat BP was measured by tail artery manometry once every 4 weeks. Before they were sacrificed, the rats were weighed, and blood samples were taken. From these samples, the serum was separated by centrifugation (12,000 × g at 4 °C for 20 min) and stored at −80 °C before analysis. After the rats were sacrificed, both kidneys were immediately excised and weighed, and then each kidney was cut in half along the coronal plane. Two sections of each excised kidney were stored at −80 °C for western blot analysis. The remaining sections were fixed in 4% buffered paraformaldehyde for 72 h at 4 °C, then transferred into 0.5% buffered paraformaldehyde and embedded in paraffin for histopathological observation and immunohistochemical analysis.

2.4. Assessments of renal function and serum changes

The levels of serum albumin, creatinine and calcium were measured in a Cobas® 8000 modular analyser (Roche Diagnostics, Basel, Switzerland). The level of serum Vitamin D was measured in a Cobas® 6000 modular analyser (Roche Diagnostics).

2.5. Histopathological observation

The pathological changes in the kidney were examined by periodic acid-Schiff (PAS) staining. One-fourth of each kidney was immersion-fixed in 0.5% buffered paraformaldehyde and embedded in paraffin to be further examined under a light

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