Pharmacological Reports 70 (2018) 917–929

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original article

Nephroprotective effects of nebivolol in 2K1C rats through regulation of the kidney ROS-ADMA-NO pathway

Yan Wang*[,1], Mengzhen Niu[1], Sha Yin, Fei Zhang, Ruizan Shi

Department of Pharmacology, Shanxi Medical University, Taiyuan, Shanxi Province, China

ARTICLE INFO

Article history:
Received 13 November 2017
Received in revised form 25 March 2018
Accepted 12 April 2018
Available online 13 April 2018

Keywords:
2K1C hypertension
Kidney
Nebivolol
ROS-ADMA-NO pathway

ABSTRACT

Background: To evaluate the protective effect of nebivolol against kidney damage and elucidate the underlying mechanism in a two-kidney, one-clip (2K1C) rat model.
Methods: 2K1C rats were obtained by clipping left renal artery of male Wistar rats and were considered hypertensive when systolic blood pressure (SBP) was ≥160 mmHg 4 weeks after surgery. The 2K1C hypertensive rats were divided into untreated, nebivolol (10 mg/kg, *ig*), and atenolol (80 mg/kg, *ig*) treatment groups. The treatments lasted for 8 weeks. SBP, kidney structure and function, plasma and kidney angiotensin (Ang) II, nitric oxide (NO), asymmetric dimethylarginine (ADMA), and the oxidant status were examined. Kidney protein expression of NADPH oxidase (Nox) isoforms and its subunit $p22^{phox}$, nitric oxide synthase (NOS) isoforms, protein arginine *N*-methyltransferase (PRMT) 1, and dimethylarginine dimethylaminohydrolase (DDAH) 1 and 2 was tested by western blotting.
Results: Nebivolol and atenolol exerted similar hypotensive effects. However, atenolol had little effect while nebivolol significantly ameliorated the functional decline and structural damage in the kidney, especially in non-clipped kidney (NCK), which was associated with the reduction of Ang II in NCK. Moreover, nebivolol inhibited the NCK production of reactive oxygen species (ROS) by decreasing Nox2, Nox4, and $p22^{phox}$ expression. Further, nebivolol reduced the plasma and kidney ADMA levels by increasing DDAH2 expression and decreasing PRMT1 expression. Nebivolol also increased the NCK NO level by ameliorating the expression of kidney NOS isoforms.
Conclusions: Our results demonstrated that long-term treatment with nebivolol had renoprotective effect in 2K1C rats partly *via* regulation of kidney ROS-ADMA-NO pathway.

Introduction

Renovascular hypertension (RVH), resulting from renal artery stenosis, is a very frequent form of secondary hypertension. It accounts for 1% to 2% of all cases of hypertension in the general population and for 5.8% of cases of secondary hypertension [1]. RVH patients are prone to developing resistance to traditional antihypertensive agents, which raises the incidence of and mortality from the condition. Timely application of appropriate pharmacological agents not only ensures the optimum control of blood pressure but also provides further benefits such as the prevention of ischemic nephropathy progression.

Unlike traditional β-blockers, nebivolol is a third-generation β_1-blocker combining vasodilatory and antioxidant properties, without sympathomimetic activity. Several studies have demonstrated the kidney protection of nebivolol in hypertensive kidney transplant recipients [2] and different renal disease models [3–7]. The two-kidney, 1-clip (2K1C) model of RVH has been widely used to explore the pathogenesis of RVH. Although studies have indicated that nebivolol attenuate left ventricular hypertrophy [8] and aorta remodeling [9] in 2K1C rats, the effect of nebivolol on the renal damage in this model is not clear. Meanwhile, a pilot study has demonstrated that nebivolol improved the glomerular filtration rate (GFR) and proteinuria in patients who underwent angioplasty due to renal artery stenosis [10]. Therefore, the first purpose of our study was to evaluate the effects of nebivolol on kidney injury in 2K1C. It was also noted that in the 2K1C model, beyond the direct damage of the clipped kidney (CK), the non-clipped kidney (NCK) underwent to structural changes originating from juxtamedullary resistance vessels and then progressing in the cortical part of the kidney [11]. We thus compared the effects of nebivolol on stenotic and contralateral kidneys in this study.

* Corresponding author.
E-mail address: butou1977@163.com (Y. Wang).
[1] Co-first author.

https://doi.org/10.1016/j.pharep.2018.04.004
1734-1140/

Development of kidney injury in 2K1C animal is accompanied by increased oxidative stress and reduced nitric oxide (NO) production in the kidney [12]. There are several mechanisms involved in the reactive oxygen species (ROS)/NO imbalance, including the increasing of asymmetric dimethylarginine (ADMA). In fact, ADMA is an endogenous inhibitor of nitric oxide synthesis (NOS), produced by protein arginine N-methyltransferase (PRMT) and metabolized by dimethylarginine dimethylaminohydrolase (DDAH). It has been hypothesized that ADMA could be involved in the development of nephropathies [13]. Increased ADMA has been found in the process of chronic kidney disease (CKD) [14]. By up-regulating PRMT and reducing DDAH, increased ROS resulted in ADMA elevation. ADMA further uncouples NOS isoenzymes to generate superoxide, contributing to the elevation of ROS production. Hewedy had reported that nebivolol suppressed ADMA and attenuated cyclosporine induced nephrotoxicity [15]. Our previous study has revealed that nebivolol reduced aortic ROS and regulated the aortic ADMA system in spontaneously hypertensive rats (SHR) [16]. Here, the second purpose was to explore whether the ROS–ADMA–NO pathway is involved in the kidney protection by nebivolol in 2K1C hypertensive rats.

Material and methods

Male Wistar rats (180–200 g) were purchased from the Animal Center of our university. The use and care of rats were carried out in accordance with the National Institutes of Health guidelines, and the study was approved by the Institutional Ethics Committee of our University (No. E0241/2015). During the study periods, all rats were allowed to drink tap water, and fed with a normal sodium diet (0.5% NaCl) *ad libitum*.

Induction of 2K1C hypertension

2K1C hypertension was induced by clipping the left renal artery with a silver clip (0.2 mm internal diameter). The artery in sham operation rats was isolated without clipping (n = 6). The rats were housed individually and injected daily with penicillin G (10^5 U/kg, *ip*) for 5 days after surgery. Systolic blood pressure (SBP) was measured by the tail-cuff method. Rats with SBP $\geq$ 160 mmHg at 4 weeks after surgery were determined to be hypertensive rats. Then 2K1C rats were divided into the following groups: (1) untreated hypertensive rats (n = 6); (2) nebivolol (10 mg/kg, *ig*) treated hypertensive rats (n = 6); and (3) atenolol (80 mg/kg, *ig*) treated hypertensive rats (n = 6). The treatments lasted for 8 weeks, and SBP was measured at different time points.

Sample collections

At the beginning and end of the study, rats were put in metabolic cages, with free access to drinking water, for measuring 24-h water consumption, urine volumes and sodium excretion. To avoid the degradation of protein during urine collection, protease inhibitors were added to the urine. Urine samples were centrifuged at 1000 × *g* for 5 min at 4 °C and kept at −80 °C. Then, after 8 h of fasting, the rats were anesthetized. Blood was collected from the abdominal aorta into chilled heparinized tubes and centrifuged at 1500 × *g* for 10 min at 4 °C. The serum obtained was aliquoted and kept at −80 °C. The left and right kidneys were quickly removed and weighed. Then kidney was divided into 3 parts: the first part was fixed in 4% paraformaldehyde for 24 h and embedded in paraffin blocks for histology and immunofluorescence test; the second part was frozen in liquid nitrogen for western blot; the third and last part was homogenized to obtain a 10% w/v homogenate. Then homogenates were centrifuged at 13,000 × *g* for 15 min at 4 °C, and the supernatants were used to measure NO, angiotension (Ang) II, ADMA, and the oxidant status.

Determination of biochemical parameters

Serum creatinine (Scr) and urinary creatinine (Ucr), urinary Na^+ were assayed by using an automated analyzer. The creatinine clearance rate (Ccr) was calculated according Cockcroft and Gault formula. Urine total protein (UTP) was determined by standard methods. Urinary microalbumin (mALB) was determined by an enzyme-linked immunosorbent assay (ELISA) and adjusted to per milligram of Ucr. Plasma and kidney ADMA was measured by ELISA. Blood urea nitrogen (BUN) was measured by a colourimetric method. Plasma and renal NO was measured using the Griess reagent.

Kidney histology and immunofluorescence

For histology, paraffin sections were stained respectively with hematoxylin and eosin (HE), Masson's trichrome and periodic acid-Schiff (PAS). Tubular damages were assessed on PAS-stained sections by scoring tubular cell necrosis, tubular dilatation, cast deposition and brush border loss in 10 non-overlapping fields in the cortex and corticomedullary junction. Injury was scored by on a 5-point scale: 0 = no damage, 1 = 1%–10%, 2 = 10–25%, 3 = 25–50%, 4 = 50–75%, 5 = more than 75%. The glomerulosclerosis index (GSI) was evaluated from 10 glomeruli on PAS-stained sections. The semi-quantitative score of GSI was evaluated according to the following criteria: 0, normal; 1, glomerulosclerosis area <25%; 2, 25%–50%; 3, 50%–75%; 4, >75%, and then results were averaged. To assess the degree of fibrosis, 10 fields from the cortex and 10 fields from the medulla were assessed on Masson-stained sections using the ImageJ software by counting the percentage of fibrotic areas, and the results were averaged. For immunofluorescence, after dewaxing and antigen retrieval, sections were blocked with 10% goat serum in phosphate-buffered saline with tween 20 for 1 h. Then the sections were incubated overnight at 4 °C with the following primary antibodies respectively: rabbit anti-nephrin (1:200 dilution; BM1669, BosterBio, Wuhan, China) and rabbit anti-kidney injury molecule-1 (KIM-1; 1:200 dilution, BA3537). On the next day, the sections were incubated with a secondary antibody (goat anti-rabbit Alexa Fluor-488; 1:400 dilution, ab150077, Abcam) for 1 h at room temperature and mounted in VectAshield® HardSet™ (H-1400) medium. Images were acquired with a Leica DMi8 fluorescence microscope. All images were scored in a blinded manner.

Measurement of plasma renin activity (PRA), plasma and kidney Ang II

Plasma renin activity (PRA) was analyzed using an Ang I ^{125}I radioimmunoassay kit. Levels of Ang II in the plasma and kidney were determined by ELISA (AEMKO, Beijing, China). The antibody used did not cross-react with other angiotensins or angiotensinogens.

Measurement of renal oxidative stress

Reduced glutathione (GSH), superoxide dismutase (SOD), 3-nitrotyrosine (3-NT), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA) levels in the kidneys were measured following the manufacturer's instructions. Urinary 8-iso-prostaglandin (PG) was determined by ELISA.

Western blotting

Frozen kidney tissues were homogenized and centrifuged at 13,000 × *g* at 4 °C for 20 min. The total protein content was