Vaccarin alleviates hypertension and nephropathy in renovascular hypertensive rats

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Abstract. The kidney is an important organ in the regulation of blood pressure, and it is also one of the primary target organs of hypertension. Kidney damage in response to hypertension eventually leads to renal insufficiency. The authors previously demonstrated that vaccarin exhibits a protective role in endothelial injury. However, the effects of vaccarin on the two-kidney, one clip (2K1C) renovascular hypertension model and subsequent kidney injury have yet to be fully elucidated. The present study was designed to investigate the roles and mechanisms of vaccarin in attenuating hypertension and whether vaccarin had beneficial effects on kidney injury. The 2K1C rats had greater fibrosis, apoptosis, reactive oxygen species production, inflammation, angiotensin II (Ang II) and angiotensin type 1 (AT1) receptors in the right kidney compared with normotensive rats, which were alleviated by a high dose of vaccarin and captopril. Vaccarin treatment attenuated hypertension, reduced fibrosis markers, NADPH oxidase (NOX)-2, NOX-4, 3-nitrotyrosine, tumor necrosis factor-α, interleukin 1β (IL-1β), and IL-6 protein levels and altered pro-apoptotic protein levels including caspase-3, anti-apoptosis protein B cell lymphoma (Bcl)-2 and Bcl-2 associated X, apoptosis regulator in the right kidney of 2K1C rats. These findings suggest that the protective effects of vaccarin on the right kidney in renovascular hypertension are possibly due to downregulation of fibrosis, inflammatory molecules, oxidative stress, Ang II, and AT1 receptor levels.

Introduction

Hypertension is believed to be a major reason of people deaths caused by cardiovascular diseases, which is largely responsible for chronic kidney injury and end-stage renal disease (1). Multiple therapeutic choices may slow down the development and progression of hypertensive nephropathy, a large number of hypertensive patients are still ultimately suffering to end-stage renal disease (2). The deoxycorticosterone acetate or high-salt diet-induced hypertension is associated with amplification of renal injury in Goto Kakizaki (GK) rats (3). Renal impairment is a frequent problem in cardiovascular diseases including hypertension (4). The destructive renal function contributes to tubular interstitial fibrosis, vascular sclerosis and glomerular sclerosis (5). Activation of renin-angiotensin-aldosterone system, inflammation, oxidative stress, endoplasmic reticulum stress, apoptosis and mitochondrial dysfunction are vital contributors in hypertensive nephropathy (6-9). The renal inflammation, tubular interstitial fibrosis, proteinuria and glomerular sclerosis are valuable markers for evaluation of renal dysfunction in chronic kidney disease (10). Application of angiotensin-converting enzyme inhibitor can reverse hypertension-induced proteinuria and renal damage (11). It is well accepted that antihypertensive therapy can retard the decrease in renal function (12).

Hypertension is recognized as an independent risk factor for chronic renal failure, and renal injury in response to hypertension is reflected by glomerular and tubulointerstitial damages, which is an important determinant for end-stage nephropathy and renal dysfunction (13). Complementary therapies are recommended as promising strategies for prevention and treatment of hypertension and renal damages (14-16). Vaccarin is isolated from *Vaccaria segetalis* seeds (17), which protects endothelial cells against oxidative stress or high glucose-induced injury (18,19). Bacterial cellulose and bacterial cellulose-vaccarin membranes accelerate wound healing in mice (19). We recently established that intraperitoneal injection of vaccarin ameliorate renovascular hypertension and cardiovascular remodeling in rats (20). However, it is so far unclear whether or not vaccarin can prevent the renal injury secondary to hypertension and, if yes, what were the possible mechanisms. Therefore, we assessed the effects of chronic infusion with vaccarin on renal structure in renovascular hypertensive rats and further attempted to clarify the underlying mechanisms.

Materials and methods

Animals. Male Sprague-Dawley rats weighing 160-180 g were purchased from Vital River Laboratories Co., Ltd. (Beijing, China). All experiments were conformed to the Guide for

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the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication, 8th edition, 2011). All procedures were complied with the Experimental Animal Care and Use Committee of Jiangnan University. All animals were caged in a temperature-controlled and humidity-controlled room and they were free accessed to standard chow and tap water. All rats were sacrificed under overdose of anesthesia (pentobarbital sodium, peritoneal injection) to minimize discomfort and pain.

Renovascular hypertensive models. The renovascular hypertensive models (two-kidney one-clip, 2K1C) were produced as we previously described (21,22). In short, the rats were anaesthetized by peritoneal injection of pentobarbital sodium (60 mg/kg) ip. A retroperitoneal flank incision was made to expose the right renal artery, and a U-shaped silver clip of 0.2-mm internal diameter was used to partly occlude the right renal artery under sterile techniques. The sham operated rats (Sham) rats underwent similar surgery without clipping. Two weeks after operation, the 2K1C rats received intraperitoneal injection of saline, low dose of vaccarin (10 mg/kg; Shanghai Shifeng Technology Co., Ltd., Shanghai, China), high dose of vaccarin (30 mg/kg), captopril (30 mg/kg; Beijing Inoke Co., Ltd., Beijing, China) for 14 days, respectively. The sham operated rats were treated with intraperitoneal injection of saline at the same time. The concentration of vaccarin used in the present study was determined according to our preliminary studies and other previous reports (20,23-25).

Blood pressure and heart rate measurement. The systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) measured using a a noninvasive computerized tail-cuff IITC blood pressure system (MRBP-2; IITC Life Science Inc., Woodland Hills, CA, USA) according to the manufacturer's instructions. The rats were warmed for 30 min at 28˚C in a bag before each measurement to obtain steady pulse level. The SBP, DBP, MAP, and HR were averaged by 10 measurements (26).

Angiotensin (Ang) II levels. The Ang II levels in the right kidney were determined by using an enzyme-linked immunosorbent assay (ELISA) kits (Boster Biological Technology Co., Ltd., Wuhan, China) according to the manufacturer's descriptions. The reacted microtiterplate was ended with stop solution, and the optical density was read at 450 nm with a microtiter plate reader (STNERGY/H4; BioTek Instruments, Inc., Winooski, VT, USA).

Angiotensin-converting enzyme (ACE) activity assay. The activity of ACE was determined using commercially available kits (Beijing Equation Biological Science and Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions as previously described (27,28). The activity of ACE in the right kidney was expressed in U/mg protein.

Histopathology and immunohistochemistry. The rats were sacrificed with overdose of pentobarbital sodium, the right kidney were collected, paraffin-embedded kidney sections (5 μm) were stained with Masson's trichrome staining as previous report (29). Immunohistochemistry with angiotensin type 1 (AT1) antibody (Abcam, Cambridge, MA, USA) were performed on the right kidney. The relative AT1 positive cells were quantified with the aid of ImageJ software.

TUNEL assay and ROS detection. The apoptosis of right kidney was assessed by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. In short, the sectioned kidney was stained using fluorescein-conjugated TUNEL, and the cell nuclei were stained with Hoechst staining. The TUNEL-positive cells were observed a fluorescence microscope (80i; Nikon Corporation, Tokyo, Japan). The apoptotic rate was quantified by counting TUNEL positive cells from 6 random fields and was expressed as a percentage of total cells. The kidney sections were 2',7'-dichlorofluorescein diacetate (DCFH-DA, 10 μM) as previous report (30,31). The fluorescence signals were captured with a multi-detection microplate reader, and quantified with the Image-Pro Plus 6.0 by using the same parameters. The measured fluorescence values were normalized to the fluorescence in control groups.

Real-time quantitative PCR analysis. Total RNA was obtained using TRIzol reagent. Equal RNA levels were reversed transcribed into cDNA using HiScriptQ RT SuperMix for qPCR (Vazyme Biotech Co., Ltd., Nanjing, China). The real-time quantitative PCR was conducted using ChamQ™ SYBR® qPCR Master Mix (Vazyme Biotech Co., Ltd.). The relative quantification of gene expression was calculated by using the $2^{-\Delta\Delta Ct}$ method (32). The sequences of required primers were listed in the Table I.

Western blot analysis. The protein in right kidney was extracted in RIPA lysis, and was electrophoresed, blotted, and then incubated with indicated primary antibodies at 4˚C overnight. The blots were then incubated with appropriate secondary horseradish peroxidase (HRP)-conjugated antibodies, the immunoreactive proteins were visualized by enhanced chemiluminescence (Merck KGaA, Darmstadt, Germany).

Reagents. Vaccarin (Fig. 1) was purchased from Shanghai Shifeng Technology Co., Ltd. Cell Meter™ terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) apoptosis assay kit was obtained from AAT Bioquest, Inc. (Sunnyvale, CA, USA). DCFH-DA (2',7'-dichlorofluorescin diacetate) were obtained from Sigma-Aldrich (Merck KGaA). The required sequences of paired primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The primary antibodies against caspase-3, Bcl-2 associated X (Bax), B cell lymphoma (Bcl)-2, AT1, NADPH oxidase (NOX)2, NOX4 and 3NT were purchased from Abcam. Antibodies against tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and IL-6 and HRP-labeled secondary antibodies were purchased from SANYING Biotechnology Co., Ltd. (Wuhan, China). Antibodies against GAPDH, and the horseradish peroxidase conjugated secondary antibody were purchased from Vazyme Biotech Co., Ltd. Immunohistochemistry kit and diaminobenzidine (DAB) were obtained from Boster Biological Technology Co., Ltd.

Statistical analysis. All results were defined as mean ± SD. Comparisons within two groups were made by Student's

Table I. Primers for real-time quantitative PCR analysis in rats.

Gene	Primer	Sequence	Accession no.
Collagen I	Forward	5'-GAGCCTAACCATCTGGCATCT-3'	NM-053304.1
	Reverse	5'-AGAACGAGGTAGTCTTTCAGCAAC-3'	
Collagen III	Forward	5'-AGATGCTGGTGCTGAGAAG-3'	NM-032085.1
	Reverse	5'-TGGAAAGAAGTCTGAGGAAGG-3'	
Fibronectin	Forward	5'-GTGAAGAACGAGGAGGATGTG-3'	XM-006245159.1
	Reverse	5'-GTGATGGCGGATGATGTAGC-3'	
GAPDH	Forward	5'-GGAAAGCTGTGGCGTGAT-3'	NM-017008.4
	Reverse	5'-AAGGTGGAAGAATGGGAGTT-3'	

Table II. Body weight, kidney weight at the end of the fourth week.

Variables	Sham	2K1C-Veh	2K1C-LDV	2K1C-HDV	2K1C-Captopril
BW, g	331.6±26.1	338.6±27.2	331.0±23.3	333.7±18.7	337.1±25.6
RKW, mg	840.0±40.7	280.9±53.6[a]	359.0±99.9	489.0±177.3[b]	812.4±91.2[b]
LKW, mg	848.3±59.1	1497.6±330.0[a]	1166.4±142.0	991.0±81.4[b]	900.0±102.8[b]
RKW/BW	2.5±0.2	0.8±0.2[a]	1.1±0.4	1.5±0.5[b]	2.4±0.2[b]
LKW/BW	2.6±0.2	4.4±0.8[a]	3.5±0.5	3.0±0.2[b]	2.7±0.3[b]

Values are mean ± SD. 2K1C indicates 2-kidney 1-clip; BW, body weight; RKW, right kidney weight; LKW, left kidney weight; LDV, low dose vaccarin; HDV, high dose vaccarin; and Sham, sham operated. [a]P<0.05 compared with the Sham. [b]P<0.05 compared with 2K1C-Veh.

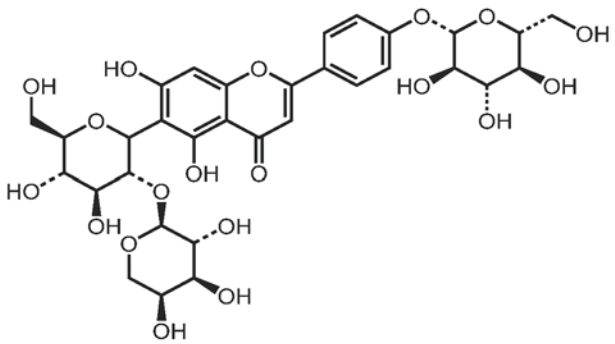

Figure 1. The chemical structure of investigated vaccarin.

t-test. Statistical analysis was performed by ANOVA/Dunnet t-test for multiple group comparisons. The criterion for statistical significance was set at P<0.05.

Results

General data. There was no significant difference in body weight (BW) between the five groups at the end of the 4th week after 2K1C or sham operation. The right kidney weight (RKW) was substantially decreased, but the left kidney weight (LKW) was obviously increased in 2K1C rats compared with those in Sham-operated rats, which were reversed by high dose of vaccarin (HDV) and captopril. The lowed RKW/BW ratio and enhanced LKW/BW ratio in 2K1C rats were restored by both high dose of vaccarin and Captopril (Table II).

Effects of vaccarin on blood pressure and heart rate in 2K1C rats. The SBP (Fig. 2A), MAP (Fig. 2B), DBP (Fig. 2C) registered on tail artery in conscious state of 2K1C rats were significantly higher than those in Sham rats four weeks after surgery. It is interesting that vaccarin at both low dose and high dose effectively reduced SBP (Fig. 2A), MAP (Fig. 2B), DBP (Fig. 2C) in 2K1C rats, but the high dose of vaccarin caused a similar effect as captopril. There was no significant difference in HR (Fig. 2D) among groups at the end of four weeks.

Effects of vaccarin on renal fibrosis in 2K1C rats. Masson's staining showed that the renal fibrosis was significantly elevated in 2K1C rats compared with Sham rats, which was consistent with previous findings (33-35). Intraperitoneally administrated with high dose of vaccarin or captopril abrogated the fibrosis in the right kidney in 2K1C rats (Fig. 3A and B), which were further confirmed by the down-regulated markers of fibrosis (collagen-I, collagen-III and fibronectin, Fig. 3C) in the right kidney of 2K1C rats.

Effects of vaccarin on renal cell apoptosis in 2K1C rats. The 2K1C rats had a greater cell apoptotic rate in the right kidney compared with normotensive rats. Compared with rats in the 2K1C group, both high dose of vaccarin and captopril suppressed renal cell apoptosis of right kidney, as revealed by TUNEL staining (Fig. 4A and B). The expressions of pro-apoptotic proteins including caspase-3 and Bax were upregulated, whereas anti-apoptosis protein Bcl-2 was decreased in 2K1C rats, which were treated by high dose of vaccarin and captopril (Fig. 4C).

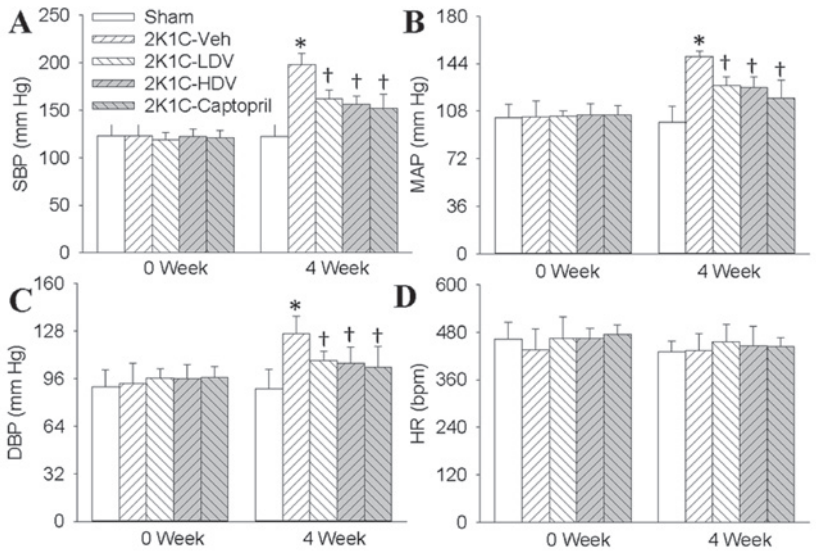

Figure 2. Effects of Vaccarin on the BP and HR in sham-operated or 2K1C rats. The 2K1C rats were subjected to intraperitoneal injection low dose of Vaccarin (LDV, 10 mg/kg/day), high dose of Vaccarin (HDV, 30 mg/Kg/day) or captopril (30 mg/Kg/day) for 14 days after 2 weeks' operation. The (A) SBP, (B) MAP, (C) DBP and (D) HR were measured with a noninvasive computerized tail-cuff system in conscious rats. Values are expressed as means ± SD. (n=7 rats/group). *P<0.05 vs. Sham; †P<0.05 vs. 2K1C-Veh (vehicle). BP, blood pressure; HR, heart rate; 2K1C, two-kidney, one clip; DBP, diastolic blood pressure; SBP, systolic blood pressure; MAP, mean arterial pressure.

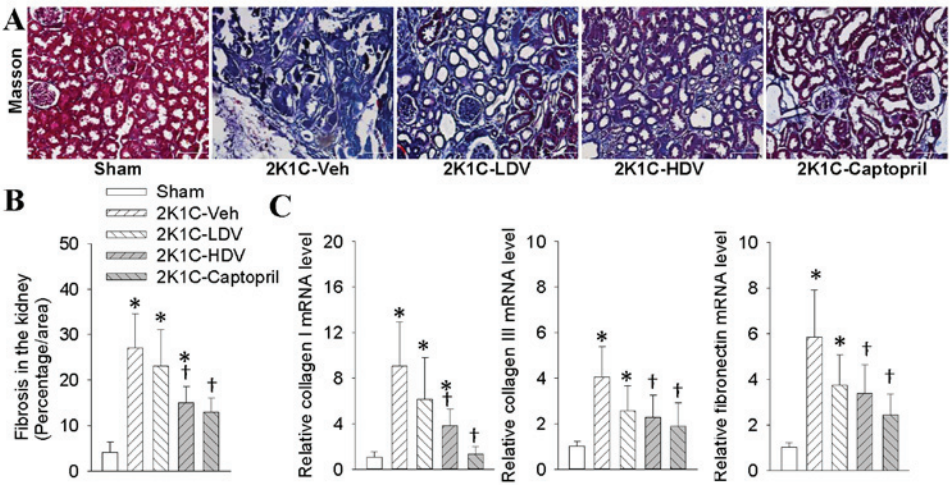

Figure 3. Effects of Vaccarin on the right renal fibrosis in sham-operated or 2K1C rats. The 2K1C rats were subjected to intraperitoneal injection low dose of Vaccarin (LDV, 10 mg/kg/day), high dose of Vaccarin (HDV, 30 mg/kg/day) or captopril (30 mg/kg/day) for 14 days after 2 weeks' operation. (A) The sectioned right kidney was collected and stained with Masson's trichrome staining (x200). (B) Bar graph showing quantitative analysis of renal fibrosis. (C) Collagen I, collagen III and fibronectin mRNA expressions in right kidney. Values are expressed as means ± SD (n=4 for each group). *P<0.05 vs. Sham; †P<0.05 vs. 2K1C-Veh (vehicle). 2K1C, two-kidney, one clip.

Effects of vaccarin on oxidative stress in 2K1C rats. Immunofluorescence revealed that 2K1C rats exhibited higher superoxide in the right kidney, as determined by fluorescent labeled DCFH-DA in comparison with Sham rats (Fig. 5A and B). When compared with Sham rats, 2K1C rats had more NOX2, NOX4, and 3NT protein levels in the right kidney, as measured by western blot analysis (Fig. 5C). Interestingly, chronic application of high dose of vaccarin and captopril prevented the increase in oxidative stress related markers in right kidney of 2K1C rats (Fig. 5).

Effects of vaccarin on inflammatory cytokines in 2K1C rats. We observed that the protein expressions of TNF-α, IL-1β and IL-6 were higher in the right kidney of 2K1C rats than those in Sham rats, both high dose of vaccarin and captopril treatment resulted in significant decreases in TNF-α, IL-1β and IL-6 protein levels in 2K1C rats (Fig. 6).

Effects of vaccarin on Ang II and AT1 receptors in 2K1C rats. Immunohistochemical results revealed that AT1 receptor positive cells were up-regulated in right kidney of 2K1C rats in comparison with Sham rats, chronic intraperitoneal infusion of high dose of vaccarin and captopril prevented the AT1 receptor positive cells in renovascular hypertensive rats (Fig. 7A and B). Ang II levels in the right kidney was augmented in 2K1C rats, which was prevented by high dose of

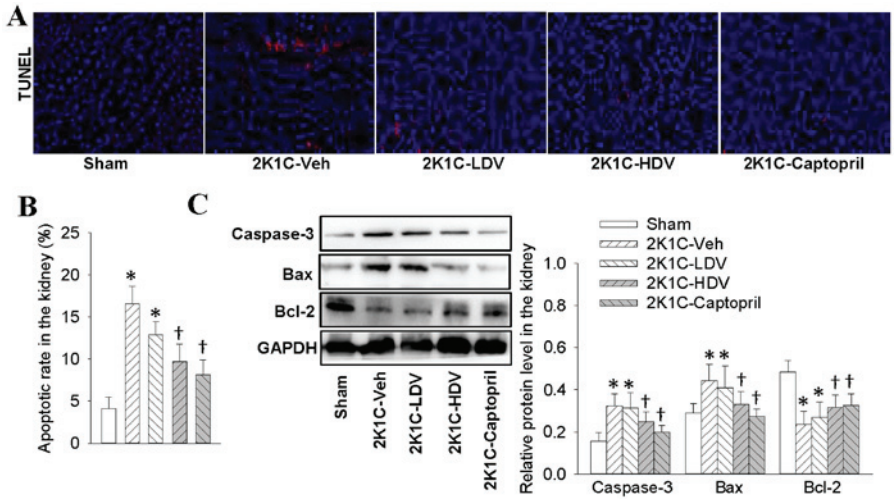

Figure 4. Effects of Vaccarin on the right renal cell apoptosis in sham-operated or 2K1C rats. The 2K1C rats were subjected to intraperitoneal injection low dose of Vaccarin (LDV, 10 mg/Kg/day), high dose of Vaccarin (HDV, 30 mg/kg/day) or captopril (30 mg/kg/day) for 14 days after 2 weeks' operation. (A) The sectioned right kidney was collected and renal apoptosis as measured or TUNEL staining (x200). (B) The numbers of TUNEL-positive cells were quantified. (C) Expressions of caspase-3, Bax and Bcl-2. Values are expressed as means ± SD (n=4 for each group). *P<0.05 vs. Sham; †P<0.05 vs. 2K1C-Veh (vehicle). 2K1C, two-kidney, one clip; TdT, terminal deoxynucleotidyl transferase; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; Bcl, B cell lymphoma.

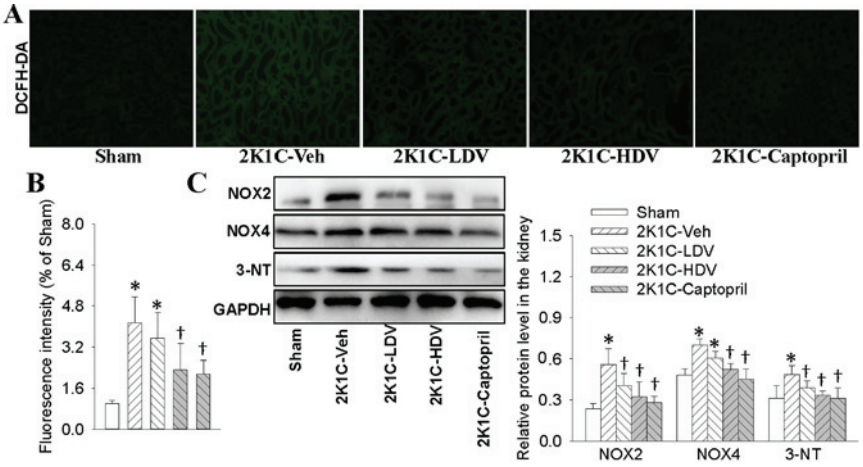

Figure 5. Effects of Vaccarin on the oxidative stress in right kidney in sham-operated or 2K1C rats. The 2K1C rats were subjected to intraperitoneal injection low dose of Vaccarin (LDV, 10 mg/kg/day), high dose of Vaccarin (HDV, 30 mg/kg/day) or captopril (30 mg/kg/day) for 14 days after 2 weeks' operation. (A) The sectioned right kidney was collected and the ROS levels measured by DCFH-DA (x200). (B) The fluorescence density were quantified and normalized to the control. (C) Expressions of NOX-2, NOX-4 and 3-NT. Values are expressed as means ± SD (n=4 for each group). *P<0.05 vs. Sham; †P<0.05 vs. 2K1C-Veh (vehicle). 2K1C, two-kidney, one clip; NOX, NADPH oxidase.

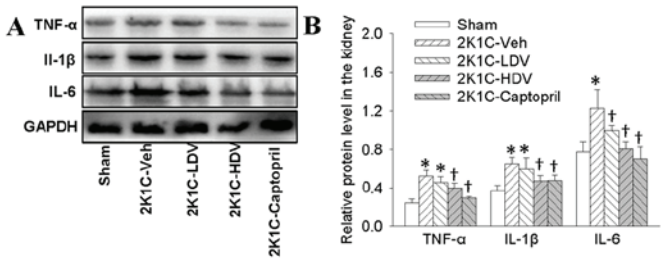

Figure 6. Effects of Vaccarin on the inflammatory response in right kidney in sham-operated or 2K1C rats. The 2K1C rats were subjected to intraperitoneal injection low dose of Vaccarin (LDV, 10 mg/kg/day), high dose of Vaccarin (HDV, 30 mg/kg/day) or captopril (30 mg/kg/day) for 14 days after 2 weeks' operation. (A) Representative blots showing the protein levels of TNF-α, IL-1β and IL-6. (B) Quantitative analysis of TNF-α, IL-1β and IL-6. Values are expressed as means ± SD (n=4 for each group). *P<0.05 vs. Sham; †P<0.05 vs. 2K1C-Veh (vehicle). 2K1C, two-kidney, one clip; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor-α.

vaccarin and captopril (Fig. 7C). Our results also showed that the ACE activity in the right kidney was higher in 2K1C rats than that in Sham rats. Similarly, vaccarin treatment attenuated the changes in the ACE activity (Fig. 7D). Western blot further demonstrated that high dose of vaccarin and captopril treatment attenuated the upregulated AT1 protein expressions in 2K1C rats (Fig. 7E and F).

Discussion

Chronic kidney disease is a prevalent medical condition with increased morbidity worldwide (36). Hypertension is an important pathogenic factor that participates in the deterioration of renal function, and many patents with hypertension eventually develop chronic kidney disease (37). Hypertension is one of the

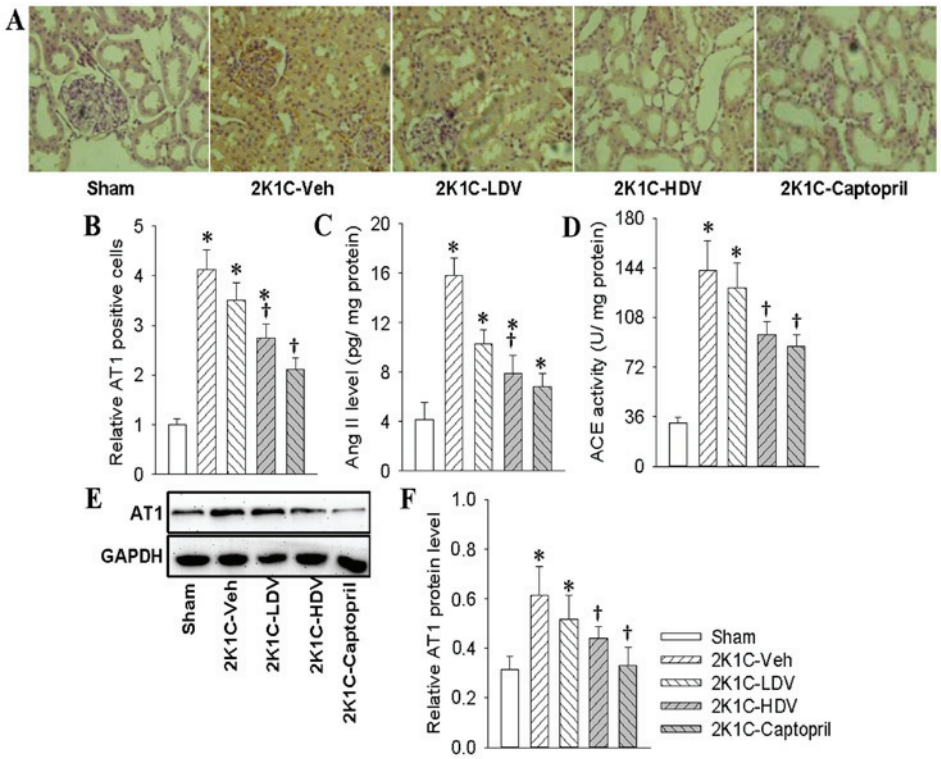

Figure 7. Effects of Vaccarin on the Ang II and AT1 expressions in right kidney in sham-operated (Sham) or 2K1C rats. The 2K1C rats were subjected to intraperitoneal injection low dose of Vaccarin (LDV, 10 mg/kg/day), high dose of Vaccarin (HDV, 30 mg/kg/day) or captopril (30 mg/kg/day) for 14 days after 2 weeks' operation. (A) The AT1 immunoreactivity in right kidney of Sham rats and 2K1C rats (x400). (B) Bar graph showing the relative cells with AT1 immunoreactivity in right kidney of Sham and 2K1C rats. (C) The Ang II levels were measured with ELISA. (D) ACE activities in right kidney were measured. (E and F) AT1 protein expressions were measured by western blot analysis. Values are expressed as means ± SD (n=7 for each group). *P<0.05 vs. Sham; †P<0.05 vs. 2K1C-Veh (vehicle). Ang II, angiotensin II; 2K1C, two-kidney, one clip; ACE, angiotensin converting enzyme; AT1, angiotensin type 1.

most common comorbidities in patients with chronic kidney disease, more than 85% of patients with stage 3-5 chronic kidney disease are complicated with hypertension (38). Vaccarin is believed to play a protective role in endothelial dysfunction. However, the therapeutic effects of vaccarin on hypertensive kidney damage have not been researched. In this study, we demonstrated for the first time that vaccarin not only normalized blood pressure, but also attenuates renal fibrosis, renal cell apoptosis, oxidative stress, inflammatory response, Ang II and AT1 receptors expressions in 2K1C renovascular hypertensive rats. These results suggested that vaccarin exerted antioxidant, antifibrotic, antiapoptotic and anti-inflammatory effects in hypertensive renal injury associated with downregulation of Ang II/AT1 system in 2K1C rats.

Renal fibrosis is one of the characteristics in end-stage renal failure, which may result from hypertension and diabetic nephropathy (39). Progressive destructive function of kidney is associated with tubular interstitial fibrosis in hypertension. It is reported that gene deletion of growth arrest-specific protein 6 lowed renal inflammation and renal fibrosis (40). The renal apoptosis is enhanced in the right kidney in 2K1C rats (41,42). Administration of angiotensin- (1-7) improves glomerular tuft volume and decreases tubular apoptosis in Akita mice (43). In this study, our data showed that vaccarin caused reductions in collagen synthesis in right kidney of 2K1C rats. Furthermore, the increased renal cell apoptosis rate evidenced by TUNEL staining, augmented pro-apoptotic proteins including caspase-3, Bax, and downregulated anti-apoptosis protein Bcl-2 in 2K1C rats were treated by vaccarin treatment. These results suggested that vaccarin may be beneficial to kidney via targeted inhibition of renal fibrosis and apoptosis.

Oxidative stress plays a key role in renal dysfunction and hypertension (44-46). Excessive ROS production response to oxidative stress may affect all types of intrinsic kidney cells (47). Oxidative stress leads to podocyte apoptosis and subsequent segmental glomerulosclerosis, resulting in kidney damage in hypertension (48). In addition, oxidative stress also induces the myofibroblasts accumulation in the kidney and remodeling of the extracellular matrix of the tubulointerstitium (48). NOXs are major sources of ROS production within the kidney (49). It has been established that the increased levels of medullary NOX2 and tissue ROS in Dahl salt-sensitive (SS) hypertensive rats are alleviated by chronic medullary interstitial infusions of NOX inhibitor apocynin (50). NOX4 is also abundantly expressed in the kidney, and knock out of NOX4 blocked oxidative stress, protein matrix production, and kidney injury in the kidney of SS rat (51). 3-NT is taken as a marker for peroxynitrite-evoked oxidative stress (52). In the present study, we showed that the ROS, NOX2, NOX4 and 3NT expressions were significantly increased in the 2K1C rats compared to those in the Sham rats, However, these effects were decreased following vaccarin treatment. These results indicated that vaccarin exhibited an obvious improvement in oxidative stress injury in 2K1C rats.

Inflammation is a major player in hypertension-associated of kidney disease. Oxidative stress and inflammation in kidney

may synergistically contribute to the pathogenesis of renal injury (53). The glomerular microinflammation is occurred in persistent systemic hypertension state (54). Treatment with statin abated the renal inflammation and podocyte damage in DOCA-salt hypertension rats (55). Pharmacological inhibition of galectin-3 reduces inflammatory cytokines, and extracellular matrix proteinases, and renal fibrosis in TGR (mREN)27 (REN2) hypertensive rats (4). The levels of TNF-α are upregulated in the kidney of Ang II-dependent hypertensive models and associated kidney disease (56). Anti-inflammatory therapy may be promising therapeutic strategy for hypertensive kidney damage. Our results showed that vaccarin treatment decreased the expressions of inflammatory mediators including TNF-α, IL-1β and IL-6 in the right kidney of 2K1C rats. These data hinted that vaccarin may act anti-inflammatory effects to protect kidney from injury in 2K1C renovascular hypertensive rats.

The renin-angiotensin system (RAS) has a critical role in mammalian homeostasis (57). The RAS family consists of angiotensinogen, renin, angiotensin-converting enzyme, Ang II and AT1 receptors, and other main components, among which, Ang II and AT1 receptors displayed powerful effects on kidney damage by a variety of mechanisms in chronic kidney disease (58). Mounting evidence demonstrates that local intrarenal RAS activation may directly dictate kidney damage (59). Ang II binds to AT1 receptors to stimulate generation of reactive oxygen species, and renal inflammation, which are synergistically for the initiation and progression of renal damage in hypertension (60). It has been established that Ang II infusion leads to hypertension and renal fibrosis in mice or rats (61). The abnormal formation of Ang II triggers systemic hypertension and renal damages associated with activation of fibrogenic, pro-inflammatory and apoptotic pathways (6). AT1 receptor antagonist Losartan is used to treat hypertension and targeted renal damage. Our study showed that the elevated SBP, MAP, DBP, Ang II and AT1 receptor levels were increased in 2K1C rats in comparison with Sham rats, which were partially abolished by vaccarin in 2K1C rats. These results implied that vaccarin may counteract Ang II/ AT1 system in the kidney to impede renal fibrosis, apoptosis, ROS generation and inflammatory response, thus protecting against hypertension-related renal damages.

Taken together, our results provided for the first time that vaccarin protected the 2K1C rats from renal oxidative stress, renal inflammatory response, renal injury and hypertension. Vaccarin may be employed as a complementary and alternative pharmacological agent that ameliorates hypertension and renal function detriment in subjects who were predisposed to hypertension. Nevertheless, as a natural product, high dose of vaccarin at least showed the similar protective effects on hypertension and cardiovascular remodeling as captopril from animal research. In addition, such ACE inhibitors including captopril have clinical side effects including cough, angioneurotic edema, and deleterious effects during pregnancy (62,63). In terms of vaccarin, there are still many unsolved problems, such as whether vaccarin can be employed as a prescription for hypertension in hypertensive patients, whether combination of vaccarin with classical antihypertensive drugs exerted better effect and less adverse reactions on hypertension. Further studies are needed regarding the more detailed role of vaccarin in hypertension and cardiovascular remodeling.

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