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Renoprotective Effects of Omapatrilat Are Mediated Partially by Bradykinin

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Key Words

Angiotensin-converting enzyme inhibition · Neutral endopeptidase inhibition · Omapatrilat · Bradykinin · *L*-NAME · Glomerular dynamics · Proteinuria · Nephrosclerosis · Apoptosis

Abstract

Aim: To investigate the effects of omapatrilat on systemic and renal hemodynamics, glomerular dynamics, renal function, and histopathological changes as well as the participation of the bradykinin B2 receptor in WKY, SHR, and *L*-NAME/SHR rats. ***Methods:*** Eight groups of 17-week-old rats were examined using renal micropuncture techniques and histopathological analyses after 3 weeks of treatment: group 1, WKY control; group 2, WKY+omapatrilat (40 mg/kg/day); group 3, SHR control; group 4, SHR+omapatrilat; group 5, SHR+*L*-NAME (50 mg/l); group 6, SHR+*L*-NAME+omapatrilat; group 7, SHR+*L*-NAME for 3 weeks followed by omapatrilat for a subsequent 3 weeks, and group 8, SHR+*L*-NAME+omapatrilat+bradykinin antagonist icatibant (500 μg/kg/day). ***Results:*** In WKY and SHR, omapatrilat significantly reduced the mean arterial pressure, increased effective renal blood flow and single nephron plasma flow associated with reduced glomerular arteriolar resistances. Furthermore, omapatrilat prevented and reversed *L*-NAME induced urinary protein excretion, glomerular and arteriolar injuries, glomerular morphometric alterations, and glomerular apoptosis (at least, $p < 0.05$). Icatibant partially inhibited these beneficial effects of omapatrilat. ***Conclusion:*** Omapatrilat provided potent antihypertensive and renoprotective actions, which were mediated, in part, by bradykinin.

Introduction

Omapatrilat is a dual inhibitor of the angiotensin-converting enzyme (ACE) and of neutral endopeptidase (NEP) [1, 2]. Inhibition of both enzymes leads to a higher level of bradykinin by additive reductions in its degradation [3–6], which may provide potential benefit in hypertension and its target-organ damage [1, 2]. Prior experimental study has demonstrated omapatrilat to have a potent antihypertensive action regardless of the renin or sodium status [7], while affording cardiovascular and renal protection [8–13] and prevention of endothelial dysfunction [14–16]. Moreover, clinical trial showed that omapatrilat improved function in patients with heart failure [17, 18], although its effects on renal hemodynamics and histopathology have been less well investigated.

Therefore, we hypothesized that omapatrilat would produce beneficial effects on renal hemodynamics and histopathology, and that bradykinin might be involved in

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the responses to omapatrilat. The aim of this study was thus to determine the effects of omapatrilat on systemic and renal hemodynamics, glomerular dynamics, glomerular and arteriolar injuries, glomerular morphometric alterations, and glomerular apoptosis in normotensive Wistar-Kyoto (WKY) and chronically treated spontaneously hypertensive rats with *L*-NAME (*L*-NAME/SHR). Additionally, the contribution of bradykinin to the effects of omapatrilat was evaluated with its selective receptor antagonist icatibant.

Materials and Methods

Studies were performed on male WKY and SHR rats (aged 17 weeks), obtained from Charles River Laboratories (Wilmington, Mass., USA). All rats were maintained at 20°C in a humidity- and light-controlled room, and were housed in plastic cages where they were given standard rat chow (PMI Feeds Inc, St. Louis, Mo., USA) and tap water ad libitum. The experimental design and protocol had been approved in advance by our institutional Animal Care and Use Committee.

The rats were randomly divided into 8 groups: group 1, WKY control (n = 8); group 2, WKY+omapatrilat (40 mg/kg/day by gastric gavage for 3 weeks, n = 8); group 3, SHR control (n = 10); group 4, SHR+omapatrilat (40 mg/kg/day for 3 weeks, n = 10); group 5, SHR+*L*-NAME (50 mg/l in drinking water for 3 weeks, n = 9); group 6, SHR+*L*-NAME (50 mg/l)+omapatrilat (40 mg/kg/day for 3 weeks, n = 9); group 7, SHR+*L*-NAME (50 mg/l) for the initial 3 weeks followed by a subsequent 3 weeks treatment period with omapatrilat (40 mg/kg/day, n = 9), and group 8, SHR+L-NAME (50 mg/l)+omapatrilat (40 mg/kg/day)+ icatibant (selective bradykinin type-2 receptor antagonist, 500 μg/kg/day s.c. via osmotic minipump for 3 weeks, n = 9). *L*-NAME was purchased from Sigma Chemical Co, St. Louis, Mo., USA, and omapatrilat was supplied by Bristol Myers Squibb Pharmaceuticals (Princeton, N.J., USA). Icatibant was provided by Aventis Pharma Deutschland GmbH, and was administered subcutaneously by means of a minipump (Model 2ML4, Alza Co). Before initiating the renal micropuncture study, 24-hour urinary collections were obtained to determine protein ($U_{Prot}V$, Lowry method [19]) and sodium ($U_{Na}V$, Beckman Astra 8 flame photometer) excretions [20–22].

Micropuncture Technique

Rats were anesthetized with thiobutabarbital sodium (Inactin, 100 mg/kg, i.p.; Byk-Gulden, Constance, Germany) and placed on a heating pad to maintain rectal temperature at 37°C throughout the study. After tracheal intubation, a polyethylene catheter (PE-50) was inserted into the right femoral artery to permit intermittent blood sampling and measurement of mean arterial pressure (MAP) and heart rate. Arterial pressure was measured through a transducer (Model P23 Dd, Statham Instruments, Oxnard, Calif., USA) connected to a multichannel polygraph (Sensor Medics R612, Beckman Instruments, Schiller Park, Ill., USA). The right carotid artery was cannulated with a thermistor microprobe (Type IT-18, Physitemp Instruments Inc., Clifton, N.J., USA) connected to a thermodilution device (Cardiotherm 500, Columbus Instruments, Columbus, Ohio, USA) to determine cardiac output, which was normalized for body weight and expressed as cardiac index (CI, ml/min/kg). Total peripheral resistance (TPR) was calculated as the quotient of MAP and CI. The right jugular vein was cannulated with a PE-50 catheter for infusion of ^{3}H-methoxyinulin (850 μCi/ml) at a rate of 0.1 ml/100 g/h. The bladder was cannulated with a PE-100 catheter for right kidney urine collection. The left kidney was exposed subcostally, its renal surface was bathed in 0.9% NaCl, and its ureter was cannulated with PE-10 tubing for urine collection. The right femoral vein was cannulated with a PE-50 catheter for 12% albumin infusion during the first 45 min of surgery at a rate of 0.4 ml/100 g/h. Thereafter, saline, containing 1% albumin and 1.5% *p*-aminohippurate (PAH; Merck Sharp & Dohme, West Point, Pa., USA), was infused at a rate of 0.4 ml/100 g/h [21, 22]. After a 1-hour equilibration period, urine was collected over four 30-min periods with blood samples being withdrawn at their midpoints. Simultaneously, the following micropuncture maneuvers were made: (1) precisely timed (90 s) samples of fluid were collected from 4–6 randomly selected superficial proximal tubules to determine the single nephron glomerular filtration rate (SNGFR); (2) efferent arteriolar (P_E), proximal tubular (P_T) and stop-flow pressures (SFP) were measured directly by a servo-nulling system (Instrumentation for Physiology & Medicine, San Diego, Calif., USA), and (3) efferent glomerular arterial blood was withdrawn directly from 2 or 3 superficially located 'star vessels' [20–22]. Glomerular capillary hydrostatic pressure (P_G) was calculated from the sum of the SFP and systemic afferent colloid osmotic pressure (Π_A). The arterial plasma protein concentration (C_A) was measured refractometrically; and Π_A and efferent colloid osmotic pressure (Π_E) were calculated using the Landis-Pappenheimer equation [23]. The pressure gradient (ΔP) across the glomerular capillary wall was calculated as $\Delta P = P_G - P_T$. The transmembrane colloid osmotic pressure difference ($\Delta\Pi$) was calculated according to the equation of Deen et al. [24] as modified by Arendshorst and Gottschalk [25]. The ^{3}H-inulin radioactivity of all tubular fluid, plasma and urine samples was counted to determine SNGFR and GFR. These measurements were used to calculate afferent (R_A) and efferent (R_E) glomerular arteriolar resistances and the glomerular capillary filtration coefficient (K_f). At the termination of each study, blood was withdrawn to determine serum creatinine and uric acid concentrations using a 747-100 Analyzer (Boehringer Mannheim/Hitachi).

Renal Morphology

After fixation in 10% neutral buffered formalin and embedding in paraffin, the kidneys were cut at 2- to 3-μm thick sections and stained with hematoxylin and eosin, periodic acid-Schiff, or periodic acid-methanamine-silver. Histological examination was conducted in a blinded fashion, and glomerular (GIS) and arteriolar (AIS) injury scores were assessed as described previously [20–22]. The frequency of glomerular lesions was determined at two renal depths, superficial and juxtamedullary cortex, each obtained by serial section. GIS and AIS indicated the total injury scores in 100 glomeruli or arterioles, respectively. The glomerular areas (A_G) of the subcapsular and juxtamedullar glomeruli were measured by tracing the outlines of those glomerular capillaries having a vascular pole; and the glomerular capillary tuft area (A_T) was measured by tracing the luminal area of all capillaries existing within 1 high power field (×400) [26–28]. The glomerular cell number (N_{GC}) was counted as the total number of 3 kinds of intraglomerular cells: endothelial, mesangial, and epithelial cells. In addition, several 5-μm-thick sections from each kidney were obtained to determine apoptosis as previously described [26–28]. Glomerular cell apoptosis, as an indicator of DNA damage, was ana-

Table 1. Body and organ weights

Groups		n	Body weight g	Left ventricle mg/g	Left kidney mg/g	Aorta mg/mm/kg
1	WKY control	8	349 ± 5.7	2.4 ± 0.07	3.5 ± 0.14	2.4 ± 0.10
2	WKY+O	8	350 ± 5.9	2.2 ± 0.11	3.6 ± 0.16	2.2 ± 0.08
3	SHR control	10	355 ± 4.9	2.8 ± 0.07**	3.5 ± 0.14	2.8 ± 0.10**
4	SHR+O	10	346 ± 3.7	2.3 ± 0.03††	3.6 ± 0.04	2.5 ± 0.07†
5	SHR+*L*-NAME	9	325 ± 6.4††	3.3 ± 0.10††	3.8 ± 0.11	4.0 ± 0.10††
6	SHR+*L*-NAME +O	9	346 ± 5.7§§	2.6 ± 0.05§§	3.5 ± 0.08	2.8 ± 0.08§§
7	SHR+*L*-NAME then O	9	364 ± 4.7§§	2.5 ± 0.02§§††	3.4 ± 0.07§	2.9 ± 0.06§§
8	SHR+*L*-NAME +O+I	9	350 ± 3.9§§	2.5 ± 0.03§§	3.5 ± 0.06	2.7 ± 0.09§§

Data are means ± 1 SEM. O = Omapatrilat; I = icatibant.
* $p < 0.05$, ** $p < 0.01$ vs. group 1; † $p < 0.05$, †† $p < 0.01$ vs. group 3; § $p < 0.05$, §§ $p < 0.01$ vs. group 5.

lyzed using the CPP-32 antibody (Immunotech, Marseille, France) to the apoptosis inducer caspase-3. For quantification of CPP-32 immunohistochemistry, at least 50 glomeruli of both the subcapsular and juxtamedullary layer were assessed to calculate glomerular apoptosis scores (GAS) on a scale from 0 to 3+ by CPP-32 expression: 0 represented no expression; 1+ was up to one third expression; 2+ was one third to two thirds expression, and 3+ was more than two thirds expression. GAS indicated the total apoptosis scores in 100 glomeruli.

Statistical Analysis

All data are presented as mean ± 1 SEM. One-way ANOVA followed by Duncan's multiple range tests were used for group comparisons. A value of less than 5% was considered to be statistically significant.

Results

Body and Organ Weights

There were no differences in body weight between the control and omapatrilat treatment groups (without *L*-NAME). Left ventricular and aortic masses were significantly reduced by omapatrilat in SHR. *L*-NAME reduced body weight but increased left ventricular and aortic masses, and these changes were prevented or reversed by omapatrilat (at least $p < 0.05$; table 1).

Systemic and Whole Kidney Hemodynamics, Glomerular Dynamics and Renal Function

In WKY, omapatrilat significantly reduced MAP, increased effective renal plasma flow (ERPF) and single-nephron plasma flow (SNPF), and decreased R_A. In addition to the foregoing changes, omapatrilat significantly increased CI, decreased TPR and renal vascular resistance (RVR), and decreased R_A and R_E in SHR. The *L*-NAME severely aggravated MAP, TPR, RVR, ERPF, SNPF, R_A, R_E, and $U_{prot}V$. All of these changes were prevented and reversed by omapatrilat (at least, $p < 0.05$; table 2, 3). Furthermore, omapatrilat significantly increased urinary volume in WKY and SHR as compared with controls (table 4). Icatibant blunted the antihypertensive effect and eliminated the beneficial systemic hemodynamic effects of omapatrilat; however, it did not influence its favorable renal and glomerular hemodynamic effects in *L*-NAME/SHR.

Renal Histopathology

The GIS was significantly greater in SHR than WKY (in both subcapsular and juxtamedullary layers; however, the individual data for each are not presented). This injury was prevented by omapatrilat ($p < 0.05$; table 5). Moreover, *L*-NAME exacerbated the GIS and increased AIS. To this end, omapatrilat significantly prevented and reversed these exacerbated nephrosclerotic alterations ($p < 0.01$; table 5). Morphometrically, the A_G and A_T were significantly reduced in SHR as compared with WKY, and omapatrilat also prevented the reduced A_G. Although the A_G was not significantly different between the control SHR and the *L*-NAME/SHR (groups 3 and 5), it was increased significantly by omapatrilat in the *L*-NAME rats ($p < 0.01$; table 5). In contrast, the A_T and N_{GC} were reduced by *L*-NAME, and omapatrilat also significantly prevented and reversed these changes (at least $p < 0.05$; table 5). Icatibant significantly increased subcapsular GIS (3.0 ± 1.1 vs. 13.4 ± 6.0, $p < 0.05$), and reduced total A_T and N_{GC}. With respect to the CPP-32 labeling index, the CPP-32 scores were increased significantly in both subcapsular and juxtamedullary layers (individual data not

Table 2. Systemic and whole kidney hemodynamic responses

Groups	n	MAP mm Hg	CI ml/min/kg	TPR U/kg	ERPF ml/min/g	RVR U	GFR ml/min/g	FF %
1 WKY control	8	128 ± 3	228 ± 10	0.57 ± 0.03	3.5 ± 0.24	19 ± 1.1	1.38 ± 0.06	40 ± 2
2 WKY+O	8	106 ± 3**	248 ± 11	0.44 ± 0.03	5.3 ± 0.18**	10 ± 0.4	1.19 ± 0.09	22 ± 1.2**
3 SHR control	10	183 ± 2**	198 ± 7*	0.94 ± 0.03**	3.3 ± 0.31	29 ± 2.9*	1.30 ± 0.08	42 ± 4
4 SHR+O	10	140 ± 5††	238 ± 7††	0.59 ± 0.02††	4.6 ± 0.28††	16 ± 1.0††	1.14 ± 0.16	25 ± 2.7††
5 SHR+*L*-NAME	9	209 ± 6††	165 ± 7†	1.28 ± 0.07††	1.1 ± 0.07††	104 ± 7.0††	0.39 ± 0.07††	35 ± 4
6 SHR+*L*-NAME+O	9	172 ± 8§§	202 ± 12§§	0.88 ± 0.07§§	3.2 ± 0.30§§	29 ± 3.6§§	0.86 ± 0.10§§†	27 ± 2§§††
7 SHR+*L*-NAME then O	9	152 ± 2§§††	191 ± 9§	0.80 ± 0.04§§	4.2 ± 0.41§§†	20 ± 2.5§§†	0.88 ± 0.10§§†	21 ± 1§§††
8 SHR+*L*-NAME+O+I	9	188 ± 5¶§§	148 ± 5¶††	1.28 ± 0.06¶¶††	3.1 ± 0.20§§	32 ± 2.4§§	0.96 ± 0.16§§	30 ± 3§§††

Data are means ± 1 SEM. O = Omapatrilat; I = icatibant; MAP = mean arterial pressure; CI = cardiac index; TPR = total peripheral resistance; ERPF = effective renal plasma flow; RVR = renal vascular resistance; GFR = glomerular filtration rate; FF = filtration fraction.

* $p < 0.05$, ** $p < 0.01$ vs. group 1; † $p < 0.05$, †† $p < 0.01$ vs. group 3; § $p < 0.05$, §§ $p < 0.01$ vs. group 5; ¶ $p < 0.05$, ¶¶ $p < 0.01$ vs. group 6.

Table 3. Glomerular dynamics

Groups	n	SNGFR nl/min	SNPF nl/min	SNFF %	P_G mm Hg	R_A U	R_E U	K_f nl/s/mm Hg
1 WKY control	8	30.8 ± 3.4	104 ± 7	29.2 ± 1.8	45.2 ± 0.9	3.3 ± 0.3	1.4 ± 0.1	0.045 ± 0.006
2 WKY+O	8	32.9 ± 2.2	125 ± 8*	26.3 ± 1.1	43.8 ± 0.4	2.1 ± 0.2*	1.1 ± 0.1	0.047 ± 0.004
3 SHR control	10	27.4 ± 1.8	101 ± 7	27.4 ± 1.3	48.0 ± 0.8**	5.4 ± 0.4**	1.5 ± 0.1	0.038 ± 0.003
4 SHR+O	10	31.1 ± 2.2	142 ± 9††	22.5 ± 1.6	45.0 ± 0.5††	2.8 ± 0.3††	1.0 ± 0.1†	0.044 ± 0.004
5 SHR+*L*-NAME	9	19.3 ± 1.7†	58 ± 4††	33.6 ± 2.3††	55.8 ± 0.7††	11.0 ± 0.7††	3.4 ± 0.3††	0.017 ± 0.002†
6 SHR+*L*-NAME+O	9	27.8 ± 1.7§	103 ± 5§§	27.0 ± 0.8§§	50.4 ± 0.6§§	4.6 ± 0.2§§	1.5 ± 0.1§§	0.029 ± 0.002
7 SHR+*L*-NAME then O	9	23.9 ± 1.3	94 ± 8§§	26.3 ± 1.8§§	48.6 ± 0.4§§	4.6 ± 0.4§§	1.7 ± 0.2§§	0.028 ± 0.003
8 SHR+*L*-NAME+O+I	9	25.1 ± 1.9	101 ± 4§§	24.9 ± 1.3§§	50.3 ± 0.5§§	5.6 ± 0.2§§	1.6 ± 0.1§§	0.027 ± 0.003

Data are means ± SEM. O = Omapatrilat; I = icatibant; SNGFR = single nephron glomerular filtration rate; SNPF = single nephron plasma flow; SNFF = single nephron filtration fraction; P_G = glomerular capillary pressure; R_A = afferent arteriolar resistance; R_E = efferent arteriolar resistance; K_f = glomerular capillary filtration coefficient.

* $p < 0.05$, ** $p < 0.01$ vs. group 1; † $p < 0.05$, †† $p < 0.01$ vs. group 3; § $p < 0.05$, §§ $p < 0.01$ vs. group 5.

Table 4. Renal function

Groups	n	Creatinine mg/dl	Uric acid mg/dl	Urinary volume ml/24 h	Urinary protein mg/24 h	Urinary sodium mEq/24 h
1 WKY control	8	0.29 ± 0.05	0.49 ± 0.04	18.8 ± 0.08	20.6 ± 1.2	2.4 ± 0.4
2 WKY+O	8	0.26 ± 0.05	0.51 ± 0.05	44.9 ± 3.3**	11.9 ± 1.5	2.6 ± 0.3
3 SHR control	10	0.22 ± 0.01	0.98 ± 0.12	9.2 ± 0.7**	14.9 ± 1.0	1.8 ± 0.2
4 SHR+O	10	0.26 ± 0.03	0.95 ± 0.12	17.1 ± 1.1††	24.8 ± 1.8	2.4 ± 0.1
5 SHR+*L*-NAME	9	0.78 ± 0.17††	1.94 ± 0.64††	22.0 ± 2.5††	119.8 ± 21.1††	1.7 ± 0.3
6 SHR+*L*-NAME+O	9	0.32 ± 0.04§§	0.89 ± 0.06§§	14.7 ± 1.1§§†	16.5 ± 2.3§§	1.5 ± 0.1
7 SHR+*L*-NAME then O	9	0.32 ± 0.04§§	0.89 ± 0.06§§	14.9 ± 0.9§§†	20.3 ± 1.0§§	2.0 ± 0.1
8 SHR+*L*-NAME+O+I	9	0.37 ± 0.06§§	1.07 ± 0.13§§	13.7 ± 0.6§§†	21.4 ± 1.0§§	2.0 ± 0.1

Data are means ± 1 SEM. O = Omapatrilat; I = icatibant.

* $p < 0.05$, ** $p < 0.01$ vs. group 1; † $p < 0.05$, †† $p < 0.01$ vs. group 3; § $p < 0.05$, §§ $p < 0.01$ vs. group 5.

Table 5. Renal morphology

Groups	n	GIS/ 100 glomeruli	AIS/ 100 arterioles	A_G $\times 10^3$ μm^2	A_T μm^2	N_{GC}/ glomerulus	GAS/ 100 glomeruli
1 WKY control	8	2.67 ± 0.3	6.95 ± 1.6	18.0 ± 0.3	112.8 ± 2.2	39.6 ± 2.0	5 ± 1
2 WKY+O	8	1.28 ± 0.4	3.77 ± 0.7	18.4 ± 0.3	113.6 ± 2.4	37.7 ± 0.7	3 ± 1
3 SHR control	10	8.31 ± 0.9*	6.84 ± 1.5	16.5 ± 0.3**	102.6 ± 2.1**	42.4 ± 1.0	21 ± 5**
4 SHR+O	10	3.91 ± 0.4†	4.33 ± 1.7	20.9 ± 0.6†	106.9 ± 1.7	43.0 ± 1.5	10 ± 2††
5 SHR+*L*-NAME	9	42.9 ± 5.7††	113.2 ± 19.6††	14.6 ± 0.2	70.7 ± 2.0††	37.8 ± 0.9†	51 ± 12†
6 SHR+*L*-NAME+O	9	5.3 ± 1.1§§	10.0 ± 1.2§§	19.5 ± 0.4§§	95.1 ± 3.4§§	48.7 ± 1.4§§	22 ± 4§
7 SHR+*L*-NAME then O	9	9.0 ± 1.6§§	13.6 ± 2.9§§	21.1 ± 0.3†§§	92.3 ± 2.5§§	50.2 ± 1.3§§	18 ± 3§§
8 SHR+*L*-NAME+O+I	9	14.2 ± 5.9§§	14.6 ± 3.3§§	18.2 ± 0.3§§	75.7 ± 2.4¶†	44.0 ± 1.4¶§§	56 ± 8¶¶††

Data are means ± 1 SEM. O = Omapatrilat; I = icatibant; GIS = glomerular injury score; AIS = afferent arteriolar injury score; A_G = glomerular area; A_T = glomerular tuft area; N_{GC} = glomerular cell number; GAS = glomerular apoptosis score.

* $p < 0.05$, ** $p < 0.01$ vs. group 1; † $p < 0.05$, †† $p < 0.01$ vs. group 3; § $p < 0.05$, §§ $p < 0.01$ vs. group 5; ¶ $p < 0.05$, ¶¶ $p < 0.01$ vs. group 6.

presented) in the SHR compared to the WKY, and were decreased by omapatrilat in SHR (at least, $p < 0.05$; table 5). Furthermore, the co-treatment and post-treatment with omapatrilat significantly inhibited the *L*-NAME-induced increase of CPP-32 scores in total glomeruli (at least $p < 0.05$; table 5). However, icatibant significantly increased the CPP-32 scores in total glomeruli ($p < 0.01$; table 5).

Discussion

The results of this study demonstrate that omapatrilat was effective in reducing MAP, and it improved systemic and renal hemodynamics as well as glomerular dynamics in WKY and SHR rats. Moreover, omapatrilat not only prevented but also reversed *L*-NAME-induced severe nephrosclerosis, glomerular and arteriolar injuries, glomerular morphometric alterations as well as glomerular apoptosis. Of particular interest, bradykinin participated, at least in part, in reducing MAP and in improving systemic hemodynamics and renal histopathology, but it did not influence renal and glomerular hemodynamics in the *L*-NAME/SHR.

It is well known that the renin-angiotensin-aldosterone, natriuretic peptide, and kallikrein-kinin systems have counterbalancing effects in regulating arterial pressure and fluid volume [29, 30]. The mechanisms for this dual enzymatic inhibition may involve: decreased angiotensin II generation and increased bioavailability of bradykinin by ACE inhibition; and increased levels of natriuretic peptide, bradykinin and other vasoactive peptides by NEP inhibition [1, 2]. Omapatrilat has been shown to reduce arterial pressure regardless of the experimental and clinical renin or sodium status [7]. As compared with an ACE inhibition alone, omapatrilat seems to provide a greater antihypertensive effect [31]. In addition to this antihypertensive action, omapatrilat has produced cardiovascular, renal and endothelial protection in hypertension and heart failure [8–18]; and these effects may be superior to either ACE or NEP inhibition alone [9, 11–13, 15–17]. Our study clearly showed the favorable effects of omapatrilat on systemic and renal hemodynamics, glomerular dynamics and renal histopathological changes, thereby providing further evidence of target-organ protection. To our knowledge, this study is the first that combines the investigation of glomerular dynamics and histopathology in WKY and SHR rats and *L*-NAME/SHR with omapatrilat. An ACE inhibitor was not included for comparison with omapatrilat since we have already described the effects of ACE inhibitors (i.e., quinapril, enalapril) on renal pathophysiology in this same experimental model [20–22, 32, 33]. In WKY, quinapril significantly reduced MAP and R_A, but no other renal hemodynamic and glomerular dynamic effects were observed [32]. In contrast, omapatrilat demonstrated significantly increased ERPF and decreased FF associated with a significant increase in SNPF. In SHR and the *L*-NAME/SHR, the renoprotective effects of omapatrilat were concordant with the results of our previous reports with ACE inhibitors [20–22, 32, 33]. However, of particular note, the present study demonstrated that omapatrilat also inhibited glomerular morphometric alterations (including A_G, A_T and N_{GC}) and glomerular apoptosis (CPP-32 scores) in

the *L*-NAME/SHR. Those effects were not investigated with other ACE inhibitors.

Prolonged nitric oxide synthase inhibition has been shown to activate both the systemic and local renal renin-angiotensin systems [27, 34–36], which are considered to contribute the pathogenesis of renal injury in the *L*-NAME-treated rat model. The present study and our previous studies [20–22, 27, 28, 37] have demonstrated that impaired glomerular dynamics (glomerular hypertension) and severe hypertensive nephrosclerosis in association with a reduced glomerular tuft area, glomerular cell loss and apoptosis were induced in young SHR by prolonged *L*-NAME administration. The irregular glomerular capillary tuft area could be a major factor of hypertensive glomerular injury resulting from the increased glomerular hydrostatic pressure [27, 37]. Moreover, glomerular cell apoptosis induced by angiotensin II [27, 38] may also account for glomerular cells loss [39, 40], thereby exacerbating glomerular dysfunction. Therefore, it appears that the beneficial renal pathophysiological effects of omapatrilat were likely achieved through the diminished angiotensin II generation by ACE inhibition.

Inhibition of both ACE and NEP produces a greater bradykinin level due to their additive effects in reducing bradykinin degradation [3–6], which, in turn, may provide a potential benefit in hypertensive disease and its target-organ damage because of the vasodilating action of bradykinin [1, 2]. To evaluate the contribution of bradykinin to the observed renoprotection, the specific and selective bradykinin type-2 receptor antagonist icatibant was co-administered during omapatrilat treatment in *L*-NAME/SHR. Our results demonstrated that icatibant blunted the antihypertensive effect, eliminated the beneficial systemic hemodynamic effects, and attenuated the improved renal histopathological alterations (including subcapsular glomerular injury scores, glomerular tuft area and glomerular cell number, total glomerular apoptosis scores) although it did not influence the favorable renal and glomerular hemodynamic effects of omapatrilat in the *L*-NAME/SHR. These findings suggest that the renal and glomerular hemodynamic effects of omapatrilat were not mediated through bradykinin, although bradykinin participated, in part, in improving renal histopathology. Therefore, bradykinin seems to have improved the renal histopathological alterations independent of its local hemodynamic effects. However, it is appropriate to mention that the previously reported beneficial effects of ACE inhibition on both renal hemodynamics and histopathology (renal injury scores) were not achieved through inhibition of bradykinin degradation [22]. This earlier report suggested that the reduction in angiotensin II, rather than increased bradykinin, was the most likely explanation for the renal protective effects of ACE inhibition alone. The discrepant effect of bradykinin on renal histopathology between our present and previous findings could very well be attributed to differences in the quantitative amounts of bradykinin generated by omapatrilat and an ACE inhibitor. This possibly higher amount of bradykinin produced by ACE and the NEP inhibition in the present study could be responsible, in part, for the improved renal histopathology that we observed.

In the present study, we have shown that omapatrilat significantly reduced the degree of apoptosis of glomerular cells in SHR and *L*-NAME/SHR. Furthermore, omapatrilat plus the bradykinin B2 antagonist, icatibant, produced an increase in the apoptosis of glomerular cells (chiefly endothelial cells) with glomerular damage, and morphologically glomerular tuft narrowing and decreased glomerular cell numbers. The anti-apoptotic function of omapatrilat was completely blocked, and its hypertensive glomerular injury reduction ability was partially blocked by icatibant, suggesting the existence of bradykinin-dependent and independent functions of omapatrilat. Yoshida et al. [41] reported that kallikrein gene delivery protected against myocardial infarction and myocyte apoptosis in ischemia/reperfusion injury via the kinin-cGMP signal pathway. They suggested that the binding of cardiac kinin to bradykinin B2 receptors activated second messengers such as nitric oxide and cGMP and thus promoted inhibition of apoptosis in their model. Other studies suggested that exogenous nitrous oxide could inhibit caspase-3-like activity, and thereby prevented tumor necrosis factor-α-induced apoptosis in endothelial cells [42, 43]. Wang et al. [44] reported that another ACE inhibitor temocaprilat plus icatibant activated myocyte apoptosis in an ischemia/reperfusion injury model, also suggesting the relation between bradykinin B2 and the ERK pathway [45], although activation of the ERK pathway had been shown to play a protective role against apoptosis in vitro [46]. Taken together, our present findings suggest that the intrarenal kallikrein-kinin system plays a protective role against glomerular cell apoptosis and hypertensive glomerular injuries.

In addition, omapatrilat has the potential to augment circulating or tissue levels of natriuretic peptide through NEP inhibition [1, 2]. One recent report demonstrated that the natriuretic peptide played an important role in the cardiorenal and humoral actions of omapatrilat [11]. In contrast, another study, assessing the effects of NEP inhibition on myocardial ischemia/reperfusion injury, demonstrated that the natriuretic peptide was unlikely to

account for the cardioprotective effect of the NEP inhibitor [47]. Our findings documented a natriuretic response to omapatrilat, as evidenced by significantly increased urinary volume and a trend of increase of urinary sodium excretion in WKY and SHR. However, the issue concerning the contribution of the natriuretic peptide in renoprotection still remains to be established.

In conclusion, our findings have demonstrated that omapatrilat resulted in potent antihypertensive and renoprotective effects in the WKY, SHR, and the *L*-NAME/SHR. Inhibition of bradykinin degradation seemed to participate, at least partially, in lowering arterial pressure and in producing renoprotection in the *L*-NAME/SHR model.

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