Beneficial and adverse renal and vascular effects of the vasopeptidase inhibitor omapatrilat in renovascular hypertensive rats

Ulrich O. Wenzel¹, Gunter Wolf¹, Ivonne Jacob¹, Christian Schwegler¹, Arish Qasqas¹, Kerstin Amann³, Udo Helmchen² and Rolf A. K. Stahl¹

¹Department of Medicine, Division of Nephrology and ²Department of Pathology, University Hospital Hamburg-Eppendorf and ³Department of Pathology, University Hospital Erlangen, Germany

Abstract

Background. Vasopeptidase inhibitors are a new class of compounds that inhibit both angiotensin-converting enzyme (ACE) and neutral endopeptidase. This study determined whether treatment with the vasopeptidase inhibitor omapatrilat (OMA) produced different effects on renal and cardiovascular structure compared with inhibition of ACE by enalapril (ENP) in rats with two-kidney, one clip hypertension (2K1C).

Methods. Hypertensive 2K1C rats were randomized into four groups and studied for another 8 weeks: no treatment, OMA, ENP or ENP combined with the diuretic hydrochlorothiazide (ENP + HCTZ). Albuminuria, vascular and renal histology as well as glomerular expression of transforming growth factor-β (TGF-β) were determined at the end of the experiment.

Results. OMA decreased blood pressure slightly better than ENP. However, combination of ENP with a diuretic lowered blood pressure equally effective as OMA. OMA was numerically more efficient in reducing cardiovascular and renal hypertensive changes compared with ENP. In contrast, the combination of ENP + HCTZ was as efficient as OMA. However, OMA lowered overexpression of TGF-β in the non-clipped kidney better than ENP or ENP + HCTZ. Antihypertensive therapy surprisingly decreased renal function as shown by increased plasma creatinine and urea and decreased creatinine clearance.

Conclusion. OMA is marginally more potent compared with ENP or ENP + HCTZ. Therefore, vasopeptidase inhibition is not superior to ACE inhibition in the prevention of cardiovascular and renal damage Goldblatt hypertension.

Keywords: ACE inhibitor; enalapril; glomerulosclerosis; omapatrilat; renovascular hypertension; vasopeptidase inhibition

Introduction

Angiotensin-converting enzyme (ACE) inhibition is a well-established treatment for hypertension and slows progression of renal disease [1]. Vasopeptidase inhibitors are a new class of drugs comprising single molecules that simultaneously inhibit both ACE and neutral endopeptidase (NEP). The later enzyme catabolizes several vasodilator molecules, including the natriuretic peptides and adrenomedullin and bradykinin [2]. As natriuretic peptides are degraded by NEP, treatment with vasopeptidase inhibitors is not only associated with reduced production of the vasoconstrictor angiotensin II but also accumulation of the aforementioned vasodilators.

Prolonged ACE inhibition induces severe tubulointerstitial damage in the clipped kidney in renovascular hypertension because function and structure of the clipped kidney are angiotensin II-dependent [3,4]. The effect of vasopeptidase inhibition on the clipped kidney is unknown. As natriuretic peptides are known to exert haemodynamic and non-haemodynamic effects that may contribute to nephroprotection [5,6], a more positive influence of omapatrilat (OMA) on the integrity of the clipped kidney compared with ACE inhibition may be expected.

Chronic two-kidney, one-clip hypertension (2K1C) Goldblatt hypertension is only in part an angiotensin II-dependent model of hypertension. Therefore, anti-
hypertensive treatment with the ACE inhibitor enalapril (ENP) does not lower blood pressure to normotension particularly in the later phase of the disease and does not prevent glomerular damage completely [4]. There is evidence that vasopeptidase inhibitors might be able to provide better haemodynamic and target-organ protection effects than ACE inhibitors in low- and high-renin models of hypertension [7–10]. However, Quaschning et al. [11] recently found less improvement of glomerulosclerosis by OMA compared with an ACE inhibitor in Dahl salt-sensitive rats, a model of hypertension with suppressed renin–angiotensin system. We therefore sought to compare the effects of the vasopeptidase inhibitor OMA with those of the ACE inhibitor ENP in rats with 2K1C Goldblatt hypertension with a special emphasis on hypertension induced cardiovascular and renal injury.

Subjects and methods

Studies were performed in 84 male Sprague–Dawley rats (Charles River, Kisslegg, Germany). Rats had free access to standard rat chow containing 0.25% sodium (Ssniff, Germany). In rats weighing 120–140 g, 2K1C hypertension was induced as described previously [4]. For this purpose, a U-shaped silver clip (0.23–0.25 mm internal diameter) was placed around the right renal artery in 74 rats through a loin incision, while the rat was under ketamine/xylazin anaesthesia (100/10 mg/kg i.m.). One rat died during surgery. Only those rats with systolic blood pressure >160 mmHg 6 weeks after surgery were included in the protocol. Four of 74 clipped rats did not develop hypertension, 16 clipped rats died within 6 weeks after surgery due to severe hypertension. Antihypertensive therapy started 6 weeks after clamping of the renal artery. Ten normotensive and the remaining 53 hypertensive rats were studied in five groups: (i) normotensive control animals (n = 10); (ii) 2K1C hypertensive animals (n = 21); (iii) 2K1C hypertensive animals + OMA (530 mg/l drinking water, n = 11); (iv) 2K1C hypertensive animals + ENP (200 mg/l, n = 10) (MSD Sharp & Dohme, Germany); (v) 2K1C hypertensive animals + ENP (200 mg/l) + hydrochlorothiazide (HCTZ) (50 mg/l, n = 11).

The study was carried out in two complete sets of experiments. Water consumption of individual rats treated with antihypertensive drugs was measured two times in the first set and five times in the second set of experiments. Mean water consumption was 79 ± 4 ml/kg body weight/day in ENP + HCTZ treated rats resulting in a consumption of 42 ± 2 mg OMA and 20 ± 1 as well 18 ± 1 mg ENP/kg body weight/day. The molecular weights of ENP and OMA are 493 and 409, respectively. 40 mg/kg/day OMA is a dose that exerts a blood pressure-lowering effect equivalent to that of 10 mg/kg/day ENP [12]. To avoid underdosing of the ACE inhibitor, an ENP concentration of 200 mg/l was used from the beginning of the experiment. The American Physiological Society’s guidelines of experimental animal research were followed and approval was obtained from the governmental and University animal care committee. A detailed analysis of the fate and the number of animals studied in the present series is given in the Subjects and methods and Results sections.

Systolic blood pressure and albuminuria

Systolic blood pressure was measured by tail cuff plethysmography in awake rats as described [4,12]. As rats are night-active and take most of the drug at night-time blood pressure measurements were performed in the morning at 8.00 a.m. and in the afternoon at 6.00 p.m. No significant differences were found. The animals were placed in individual metabolic cages and 24-h urine collections were made for determination of creatinine and albuminuria. Albuminuria was measured with a commercial ELISA (WAK Chemie, Bad Soden, Germany) as described by the manufacturer.

Renal morphology

At the end of the experiment blood was drawn for measurement of plasma creatinine and urea. The kidneys were removed and wet weight was measured. Kidney slices as well as arteria carotis and aorta thoracica were fixed in 4% buffered formalin, paraffin embedded, cut to 3–4 μm thick sections and stained with periodic acid Schiff (PAS). Ventricular heart weight was determined in one of the two subsets of experiments. Glomerular damage was evaluated as described earlier [4,13]. Thereby, grade 1 represents involvement of up to 25% of the glomerulus and grade 4 represents injury of 75–100% of the glomerulus. Fifty glomeruli were evaluated from each kidney. Quantification of the tubulointerstitial changes was determined by superposing a grid containing 40 sampling points on each of three photographs of non-overlapping cortical fields of each kidney stained with PAS reagent (magnification 400×). The number of points overlying interstitial space (interstitial volume index) was counted and expressed as a percentage of all sampling points. 120 points were evaluated for each kidney. Planimetric examinations of glomerular cross sectional area were performed by means of a Zeiss drawing tube in combination with a semi-automatic interactive image analysis system (Morphomat 30, Zeiss, Oberkochen, Germany) as described [4,13]. Using a serpentine movement from cortex to medulla and vice versa, the outlines of 30 consecutively encountered capillary tufts were traced manually and the mean glomerular random cross-sectional area (AG) was determined. The average glomerular tuft volume (VG) was then calculated as 

$$VG = \frac{\beta}{k}\frac{AG}{C_{12}}$$

where \( \beta = 1.38 \) and \( k = 1.1 \) are shape and size distribution coefficients, respectively [13]. Medial thickness of arteria carotis and aorta thoracica rings was determined using morphometry. Ten to 12 random measurements were made of one section that included the entire circumference of each vessel and averaged to obtain the final medial thickness. Vessel radii and wall thickness of interlobular and arcuata arteries were calculated on the basis that the internal elastic lamina (IEL) was fully distended to form a totally unwrinkled circle according to the method of Furuyama [14]. The total length of the IEL and medial area was estimated by planimetric examinations of the IEL and the outer circumference of the intra-renal arteries by means of a Zeiss drawing tube in combination with a semi-automatic interactive image analysis system. Interlobular arteries were identified as single muscular arteries within the cortex and arcuate arteries were identified along the corticomedullary junction surrounded by tubules. 12.8 ± 0.1 vessels were measured in each kidney.

Creatinine and urea were measured using an autoanalyser (Hitachi 717, Roche, Mannheim Germany).
Western blotting

Western blotting was performed as described recently [13]. Glomeruli were isolated by differential sieving and resuspended in Laemmli buffer. Samples were boiled and centrifuged. Protein concentration was determined with the protein DC-assay (Bio-Rad, Munich, Germany). To equal amounts of protein (100 mg), Laemmli buffer 2 and 1/5 staining solution (42.5% glycerol, 0.5% bromphenol blue) were added. Samples were electrophoresed on SDS–polyacrylamide gels. Proteins were electroblotted onto nitrocellulose (Hybond-ECL, Amersham, Braunschweig, Germany). The membrane was blocked with 5% non-fat dry milk in washing buffer (1/2 PBS 0.1% Tween 20). A rabbit anti TGF-β antibody (R&D Systems, Minneapolis, MN) was used in a 1:1000 dilution. The second antibody was a mouse anti-rabbit horseradish peroxidase-conjugated antibody in a 1:1000 dilution (Transduction Laboratories, Lexington, MA). Peroxidase labelling was detected with luminescence detection (ECL, Amersham) according to the manufacturer’s recommendations. To control for small variations in protein loading and transfer, membranes were washed and re-incubated with a mouse monoclonal anti-β-actin antibody (Sigma, Deisenhofen, Germany). Exposed films were scanned with Fluor-ST multi imager (Bio-Rad Laboratories, Hercules, CA), and data were analysed with the computer program Multi-Analyst™ from Bio-Rad.

Statistical analysis

Results are expressed as means ± SEM. One-way analysis of variance was performed with post hoc Scheffe test to correct for multiple comparison. Statistical significance was defined as \( P < 0.05 \).

Results

General parameters

Table 1 shows the characteristics of the animals studied. Only 16 of the initial 21 hypertensive rats without therapy (76%) survived the experiment after randomization. Survival was 100% in normotensive controls and in treated animals. In hypertensive rats hypertrophy of the non-clipped kidney and numerical atrophy of the clipped kidney occurred. Antihypertensive therapy decreased the weight of the clipped kidney.

Blood pressure

The systolic blood pressure is illustrated in Figure 1. Systolic blood pressure as measured by tail plethysmography was significantly lowered by OMA and ENP + HCTZ \((P < 0.001 \text{ vs } 2\text{K}1\text{C})\) and was no more statistically different to normotensive controls. ENP monotherapy also lowered blood pressure to normotension. However, numerically it was slightly less effective as OMA and EH. The mean systolic blood pressure values of the seven measurements during the 8 weeks of treatment were 113 ± 4 in OMA, 126 ± 3 in ENP and 118 ± 5 mmHg in EH treated rats. Blood pressure of controls averaged 120 ± 4 and of 2K1C 210 ± 6 mmHg.

Albuminuria

Albuminuria at the end of the experiment is shown in Table 2. Albuminuria increased in untreated hypertensive rats to 55 ± 15 mg/24 h. Albuminuria was reduced with all treatments as compared with untreated hypertensive rats. However, numerically it was less effective as OMA and EH. The mean albuminuria values were 14 ± 5 mg/24 h in ENP, 30 ± 5 mg/24 h in EH and 35 ± 5 mg/24 h in 2K1C treated rats. ENP lowered albuminuria to normotension.

Renal histologic finding

Evaluation of the glomerular damage of the non-clipped kidney of PAS stained sections was performed. Intact glomeruli were found in normotensive controls (Figure 2A). Glomerular injury was detected in the non-clipped kidney of untreated hypertensive rats (Figure 2B) as described by us and others in this model [4,13,15]. Almost no more glomerular damage was found in the OMA and ENP + HCTZ treated groups (Figure 2C and E), whereas few sclerotic glomerular lesion were still found in the non-clipped kidney of ENP treated rats (Figure 2D, arrow). Scoring of the described glomerular changes is summarized in Table 2. OMA and EH lowered glomerular damage significantly compared with untreated 2K1C rats \((P < 0.05 \text{ vs } 2\text{K}1\text{C})\). Although ENP lowered glomerular

### Table 1. Body weight, survival and kidney weight at the end of the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Survival (%)</th>
<th>Body weight (g)</th>
<th>Kidney weight (g)</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Clipped</td>
<td>Non-clipped</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>100</td>
<td>518 ± 19</td>
<td>1.40 ± 0.04</td>
</tr>
<tr>
<td>2K1C</td>
<td>16/21</td>
<td>76</td>
<td>473 ± 13</td>
<td>1.27 ± 0.05</td>
</tr>
<tr>
<td>OMA</td>
<td>11</td>
<td>100</td>
<td>466 ± 9</td>
<td>0.88 ± 0.07(^c)</td>
</tr>
<tr>
<td>ENP</td>
<td>10</td>
<td>100</td>
<td>476 ± 22</td>
<td>0.77 ± 0.07(^c)</td>
</tr>
<tr>
<td>ENP + HCTZ</td>
<td>11</td>
<td>100</td>
<td>433 ± 17(^b)</td>
<td>0.94 ± 0.07(^b)</td>
</tr>
</tbody>
</table>

\(^a\)P < 0.05 vs controls.
\(^b\)P < 0.01 vs controls.
\(^c\)P < 0.01 vs 2K1C.
Fig. 1. Systolic blood pressures measured in conscious rats before and after start of treatment is shown.

![Graph showing systolic blood pressure changes over time.](image)

Table 2. Albuminuria, glomerulosclerosis and glomerular volume

<table>
<thead>
<tr>
<th>Group</th>
<th>Albuminuria (mg/24h)</th>
<th>Glomerulosclerosis (score)</th>
<th>Glomerular volume Clipped right</th>
<th>Non-clipped left</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.48±0.88</td>
<td>0.05±0.01</td>
<td>1.08±0.09</td>
<td>1.08±0.09</td>
</tr>
<tr>
<td>2KIC</td>
<td>55.23±15.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.59±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96±0.05</td>
<td>1.45±0.14</td>
</tr>
<tr>
<td>OMA</td>
<td>1.07±0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.69±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.29±0.07</td>
</tr>
<tr>
<td>ENP</td>
<td>1.48±0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.13±0.02</td>
<td>0.74±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.35±0.12</td>
</tr>
<tr>
<td>ENP+HCTZ</td>
<td>1.35±0.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.09±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.65±0.06&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.22±0.08</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < 0.05 vs controls.<br>
<sup>b</sup>P < 0.01 vs controls.<br>
<sup>c</sup>P < 0.05 vs 2KIC.

Fig. 2. Glomeruli from controls revealed a normal appearance (A). A lightmicrograph of glomeruli from the non-clipped kidney demonstrating hypertensive glomerular injury is shown in (B). No more lesions were detected in OMA (C) and ENP+HCTZ (E) treated rats whereas few lesions were still found in ENP treated rats (D). Scoring of the glomerular damage evaluated by is shown in Table 2.
damage, this reduction failed to reach statistical significance ($P = 0.086$).

Glomerular hypertrophy is an important parameter contributing to the progression of chronic renal disease. As shown in Table 2, no significant changes were found for glomerular hypertrophy in the non-clipped kidney. In contrast, in the clipped kidney glomerular size was significantly lowered by OMA, ENP and ENP + HCTZ.

**Tubulointerstitial damage and glomerular volume**

It is known that tubulointerstitial damage precedes glomerular injury in the non-clipped kidney of 2K1C rats suggesting a dominant pathophysiologic role for the tubulointerstitial damage in nephrosclerosis [15]. We therefore evaluated the pattern of tubulointerstitial injury by point counting. The analysis revealed an increase of interstitial volume in the non-clipped kidney compared with normotensive controls confirming earlier reports [13,15]. After antihypertensive therapy with OMA, ENP and EH interstitial volume was no longer significant different to normotensive control rats. In contrast, after 8 weeks of antihypertensive therapy prominent and diffuse atrophy of tubules and interstitial fibrosis was found in the clipped kidneys. This led to a 342 and 305% increase of the interstitial volume index in OMA and ENP + HCTZ treated rats and a slightly smaller increase of 256% in ENP treated rats compared with untreated hypertensive rats (Figure 3A). Lightmicrographs of the changes evaluated by point counting in the clipped kidney are shown in Figure 3B–F. No tubulointerstitial abnormalities were found in normotensive controls (Figure 3B). The interstitium was modestly increased and focal tubular damage was found in the clipped kidney of hypertensive rats (Figure 3C). In contrast, diffuse interstitial widening and infiltration as well as severe tubular atrophy and dilatation was found in OMA (Figure 3D) and ENP + HCTZ (Figure 3F) treated rats. ENP treated rats showed less severe tubulointerstitial damage (Figure 3E).

**Renal function**

To assess overall renal function plasma creatinine, urea and creatinine clearance were measured. No significant difference was found between normotensive and hypertensive rats. However, antihypertensive therapy surprisingly decreased renal function as shown by increased plasma creatinine and urea and decreased creatinine clearance (Table 3).

**TGF-β**

We have recently demonstrated upregulation of TGF-β in 2K1C rats [13]. To further quantify the effect of antihypertensive therapy on glomerular TGF-β levels, western blot analysis of glomerular lysates was performed. Similar to our previous finding, hypertensive rats in the present study showed increased levels of TGF-β in the non-clipped kidney compared with controls (Figure 4A). A decrease of TGF-β abundance was found in the non-clipped kidney of 2K1C rats after antihypertensive therapy. Interestingly, OMA reduced TGF-β overexpression better than ENP or ENP + HCTZ.

**Cardiovascular histology**

Heart weight was determined in one of the two subsets of experiments (Table 4). Increase in heart weight was prevented by all three antihypertensive treatments.

A significant increase in media thickness was observed in arteria carotis from hypertensive rats as shown in Figure 5A. OMA, ENP and EH lowered media thickness significantly. A very similar pattern was observed for medial thickness of the aorta (data not shown). We also studied medial hypertrophy of intra-renal arteries. As shown in Figure 5B wall/lumen ratio was significantly increased in 2K1C rats. OMA, ENP as well as ENP + HCTZ decreased wall/lumen ratio significantly. However, wall/lumen ratio of the OMA treated rats was still significantly higher than in control rats.

**Discussion**

Vasopeptidase inhibition is a new concept in cardiovascular therapy. It has been shown that OMA exhibits greater antihypertensive effects than those elicited by ACE inhibitors [7]. The objective of the current study was to investigate whether vasopeptidase inhibition with OMA would offer any advantage over selective ACE inhibition in rats with Goldblatt hypertension. The results of this study suggest that treatment with OMA does only offer specific advantages in Goldblatt hypertensive rats of marginal significance.

Other studies comparing vasopeptidase inhibitors and ACE inhibitors reported greater renoprotective effects with the vasopeptidase inhibitor [8,9,10]. In two studies blood pressure was significantly higher in ACE inhibitor treated than vasopeptidase inhibitor treated rats [9,10]. Other studies reported the effects of OMA vs untreated hypertensive animals [12,16]. The data are therefore unable to distinguish between additional cardiovascular and renoprotective effects attributable to greater systemic blood pressure reduction and effects related to the unique cardiovascular and renal actions of vasopeptidase inhibition. In addition, another study in Dahl salt-sensitive rats even found less reduction of glomerular injury in OMA treated rats than ACE inhibitor treated animals [17]. Most studies also underdosed the ACE inhibitor. Anderson and co-workers used 50 mg/l drinking water ENP in their landmark study demonstrating that an ACE inhibitor limited glomerular damage in rats with renal ablation [18]. Increased protection against end-organ damage independent from blood pressure lowering was later shown by Kakinuma and Peters with increasing doses.
of ENP [19,20]. We therefore chose in the present study a dose of 200 mg/l ENP. Intake of the ACE inhibitor was 20 mg/kg/day, which is higher than in our previous studies with this model [4]. According to our experience [4] and the literature mentioned above it is therefore unlikely that higher dosing would increase the efficacy of the ACE inhibitor. Inadequate dosing of ENP might have caused the superiority of OMA over ENP in previous studies [8]. In other studies the ACE inhibitor captopril was used [11,17]. Captopril has, compared with ENP and OMA, a short halftime and might not develop a 24 h protection.

According to the law of Laplace an increased glomerular size causes an increased wall tension. The increased glomerular size in the non-clipped kidney may therefore enhance the damage caused by the increased blood pressure. As degradation of atrial natriuretic peptide (ANP) is inhibited after NEP inhibition and ANP has been shown to inhibit hypertrophy of renal cells [5], we expected to find less glomerular hypertrophy in OMA treated rats. However, a specific antitrophic effect on glomerular volume in the non-clipped kidney by OMA could not be detected. A specific antitrophic effect of OMA on media hypertrophy was recently shown in DOCA salt

Fig. 3. Analysis of cortical renal sections by point counting. Interstitial volume was significantly increased in the non-clipped kidney of untreated hypertensive rats compared with normotensive controls. Interstitial volume was no longer statistically different to controls in OMA, ENP and EH treated rats. In contrast, antihypertensive therapy increased the interstitial space in the clipped kidney dramatically (A). *P < 0.05, **P < 0.001 vs controls, #P < 0.005, ##P < 0.001 vs 2K1C. Light micrographs of the tubulointerstitial changes in the clipped kidney are shown in (B–F). Well preserved tubuli were found in controls (B). Modest tubulointerstitial damage was seen in the clipped kidney of 2K1C rats (C). Increased tubulointerstitial injury was seen in OMA and ENP + HCTZ treated rats (D and F), whereas less damage was found in ENP treated rats (E).
rats [21]. No major difference was found between OMA and ENP as well as ENP + HCTZ treated rats in different vessels indicating that ACE inhibition and blood pressure reduction are the main determinants limiting media hypertrophy in this model of hypertension.

The current status of knowledge suggests that TGF-β overexpression is a key factor for matrix accumulation. OMA clearly inhibited TGF-β overexpression better than ENP + HCTZ despite similar blood pressure control indicating that OMA has blood pressure independent TGF-β lowering action beyond ACE inhibition. A possible explanation for decreased expression of TGF-β in OMA treated rats might be greater lowering of glomerular capillary pressure [8] as it is well established that stretching force upregulates TGF-β in mesangial cells. However, the superiority in reducing TGF-β expression did not translate in better renal protection. It could therefore be possible that TGF-β does not play a major role in the development of glomerular sclerosis in the non-clipped kidney of Goldblatt rats.

ACE inhibitors have a well-known and established beneficial effect in chronic renal disease [1]. However, in the case of renal artery stenosis blockade of the renin-angiotensin system might have detri-

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Table 3. Renal function

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (mg/dl)</th>
<th>Urea-N (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.26 ± 0.01</td>
<td>20 ± 0.5</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>2KIC</td>
<td>0.33 ± 0.02</td>
<td>28 ± 2.1</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>OMA</td>
<td>0.53 ± 0.07abc</td>
<td>45 ± 3.3bd</td>
<td>1.8 ± 0.2a</td>
</tr>
<tr>
<td>ENP</td>
<td>0.48 ± 0.07a</td>
<td>36 ± 1.5a</td>
<td>1.9 ± 0.2a</td>
</tr>
<tr>
<td>ENP + HCTZ</td>
<td>0.41 ± 0.03</td>
<td>51 ± 2.3b,cd</td>
<td>1.9 ± 0.2a</td>
</tr>
</tbody>
</table>

*a P < 0.05 vs controls.  
*b P < 0.01 vs controls.  
*c P < 0.05 vs 2KIC.  
*d P < 0.001 vs 2KIC.

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Fig. 4. Expression of glomerular TGF-β protein levels. (A) A single band of ~25 kDa is visible reflecting the TGF-β dimer. Hypertensive rats showed increased levels of TGF-β in the non-clipped kidney compared with controls. This increased expression was lowered in all three treatment groups. However, OMA lowered TGF-β greater than ENP or ENP + HCTZ. The blot was washed and re-incubated with an antibody against β-actin. The glomerular lysate used in this western blot was pooled from five to six kidneys in each group. A very similar pattern was found in another western blot from the second set of experiments using again pooled proteins from five to 10 kidneys in each group. Densitometric analysis of the TGF-β bands corrected by β-actin abundance from both blots is shown below (B).
mental effects for the stenosed kidney due to renal failure. Prolonged ACE inhibition induces severe tubulointerstitial damage in the clipped kidney [3,4]. NEP catalyses the breakdown of peptides that have been shown to play a therapeutic role in the treatment of renal disease [6]. It was therefore reasonable to speculate that OMA by inhibiting degradation of nephroprotective proteins might induce less tubulointerstitial damage in the clipped kidney. However, no difference could be detected in interstitial widening as assessed by point counting in the clipped kidney between the OMA and ENP + HCTZ treated rats.

Antihypertensive therapy increased survival and decreased renal injury in the non-clipped kidney. However, surprisingly overall renal function as measured by plasma creatinine and urea as well as creatinine clearance was decreased in the antihypertensive treated groups. It is unlikely that treatments may have limited the capacity for compensatory hypertrophy in the non-clipped kidney as treatments neither decreased weights of the non-clipped kidneys nor inhibited glomerular hypertrophy in these kidneys. One reason might be the loss of function of the clipped kidney. In addition, media-lumen ratio of intra-renal arteries was numerically not completely normalized in the non-clipped kidney of antihypertensive treated rats. Stenoses of intra-renal arteries after longstanding renovascular hypertension have been described in the

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Heart weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4</td>
<td>1.17 ± 0.02</td>
</tr>
<tr>
<td>2K1C</td>
<td>9</td>
<td>1.56 ± 0.11</td>
</tr>
<tr>
<td>OMA</td>
<td>6</td>
<td>1.07 ± 0.02a</td>
</tr>
<tr>
<td>ENP</td>
<td>6</td>
<td>1.14 ± 0.05b</td>
</tr>
<tr>
<td>ENP + HCTZ</td>
<td>6</td>
<td>1.10 ± 0.03a</td>
</tr>
</tbody>
</table>

\*P < 0.01 vs 2K1C.
\*P < 0.05 vs 2K1C.

![Fig. 5. Thickness of the media of the arteria carotis was measured by planimetry. The media was increased in 2K1C rats, OMA, ENP and ENP + HCTZ lowered the thickness of the media significantly (A). *P < 0.001 vs controls, **P < 0.001 vs 2K1C. The wall/lumen ratio of the intra-renal arteries is shown in (B). The ratio was significantly increased in 2K1C rats and significantly lowered by OMA, ENP as well as ENP + HCTZ. However, statistically it was in OMA treated rats still significantly higher than in normotensive controls. In ENP and EH treated rats the wall lumen ratio was numerically higher than in controls but this difference did not reach significance. *P < 0.05, **P < 0.01 vs controls, \*P < 0.01, \*\*P < 0.001 vs 2K1C.](https://academic.oup.com/ndt/article-abstract/18/10/2005/1807491)
Vasopeptidase inhibition and renovascular hypertension

non-clipped kidney [22]. Arteriolar hypertrophy and sclerosis causing intra-renal stenoses might, at least in part, explain that the non-clipped kidney of antihypertensive treated rats was not able to compensate for the loss of the clipped kidney after ACE inhibition. Although creatinine clearance is not a perfect marker of glomerular filtration rate in the rat, creatinine is established and widely used especially in combination with plasma-urea as a reliable marker of renal function.

In summary, we demonstrate that, long-term high dose OMA lowers blood pressure marginally better in rats with Goldblatt hypertension than high dose ENP and confers slightly more cardiovascular and renal protection in the non-clipped kidney. However, if control of systemic blood pressure was closely matched by adding a diuretic to ENP, OMA and ENP + HCTZ exert very similar cardiovascular and renal effects with the exception that OMA lowered glomerular over-expression of TGF-β better than ENP + HCTZ. In contrast, OMA failed to induce less tubulointerstitial damage in the clipped kidney compared with ENP + HCTZ and antihypertensive therapy failed to improve overall renal function. The present data are confirming recent clinical data from the OCTAVE (Omapatrilat Cardiovascular Treatment Assessment Versus Enalapril) and OVERTURE (Omapatrilat Versus Enalapril Randomized Trial of Utility in Reducing Events) study showing only marginally superioritity of OMA over ACE inhibition. It has to be discussed whether the previously described superiority of OMA over ACE inhibition in different models of renal and cardiovascular disease is model dependent or due to study design favouring the vasopeptidase inhibitor.

According to published data but not to our data OMA represents an interesting alternative to lower blood pressure and to improve vascular and renal structure in experimental models of hypertension. It remains presently doubtful whether this will translate into clinical benefits of vasopeptidase inhibition.

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