Vasopeptidase Inhibition Affords Greater Renoprotection than Angiotensin-Converting Enzyme Inhibition Alone

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Abstract. The renoprotective efficacy of the vasopeptidase inhibitor omapatrilat (OMA) was compared with that of enalapril (ENA) in male Munich-Wistar rats subjected to 5/6 nephrectomy. ENA and OMA administered beginning on day 2 after surgery were equally effective in normalizing systolic BP (SBP) and preventing glomerulosclerosis (GS) for 12 wk. Micropuncture studies of rats performed using a similar treatment protocol demonstrated greater reduction of glomerular capillary hydraulic pressure with OMA than with ENA, at similar mean arterial pressures. To investigate whether these glomerular hemodynamic differences might be associated with differences in chronic renoprotective efficacy, additional rats were included in a protocol in which treatment was delayed until 4 wk after surgery (after the onset of hypertension and proteinuria) and continued for a longer period. Both treatments normalized SBP, but OMA resulted in more sustained reduction of proteinuria than did ENA. At week 20, OMA- and ENA-treated rats exhibited less GS than did untreated (control) rats at week 12, but only the difference in control versus OMA values was statistically significant [GS scores: control (12 wk), 36 ± 4%; ENA (20 wk), 22 ± 6%; OMA (20 wk), 14 ± 2%]. The remaining ENA-treated rats were euthanized at 32 wk because of rapidly increasing proteinuria, whereas the remaining OMA-treated rats demonstrated a substantially slower increase in proteinuria until euthanasia at 50 wk. At this extremely late time point, OMA-treated rats exhibited GS scores similar to those of ENA-treated rats at 32 wk and control rats at 12 wk [GS scores: ENA (32 wk), 34 ± 5%; OMA (50 wk), 38 ± 8%]. It is concluded that, in this model, OMA affords greater long-term renoprotection than ENA when doses are adjusted to yield equivalent control of SBP.

Treatment with angiotensin-converting enzyme (ACE) inhibitors (ACEI) has been demonstrated, in experimental and clinical studies, to significantly slow the progression of chronic renal disease (CRD) and therefore represents the standard with which novel renoprotective therapies must be compared (1). In clinical studies, however, ACEI treatment slows rather than arrests the progression of CRD (2–5). Therapies that provide renoprotection in addition to that achieved with ACEI alone would therefore be desirable.

Vasopeptidase inhibitors (VPI) are a new class of drugs comprising single molecules that simultaneously inhibit both ACE and neutral endopeptidase (NEP). The latter ectoenzyme is localized principally in the brush border membrane of renal tubule cells and catalyzes several vasodilator molecules, including the natriuretic peptides and adrenomedullin and bradykinin (6,7). Therefore, VPI treatment is associated with reduced production of the vasoconstrictor angiotensin II and accumulation of the aforementioned vasodilators. In experimental (8,9) and clinical (6) studies, VPI have been demonstrated to be effective antihypertensive agents in both low- and high-renin states. In addition, VPI treatment was associated with better survival rates, compared with ACEI alone, in an experimental model of cardiac failure (10), and VPI at doses that inhibit both ACE and NEP produced greater functional and hemodynamic improvement among patients with cardiac failure than did ACEI at a low dose that inhibits only ACE (11).

Although they seem to act at multiple levels to slow CRD progression, the renoprotective effects of ACEI are closely associated with their ability to normalize the elevated glomerular capillary hydraulic pressures (P<sub>GC</sub>) observed in the remaining nephrons after loss of more than one-half of the total renal mass (12). Because VPI alter the levels of multiple vasoactive peptides, it is difficult to predict the effects of VPI treatment on glomerular hemodynamics. Short-term studies in experimental models of CRD suggested that VPI treatment may afford renoprotection in addition to that achievable with ACEI alone (13,14). We thus sought to compare the renal hemodynamic and long-term renoprotective effects of the VPI omapatrilat (OMA) with those of the ACEI enalapril (ENA), in an experimental model of CRD progression.

Materials and Methods

Short-Term Treatment Protocol

Twenty-seven male Munich-Wistar rats obtained from Simonsen Laboratories (Gilroy, CA) were subjected to renal mass ablation,
under pentobarbital anesthesia (Nembutal, 50 mg/kg, intraperitoneally; Abbott Laboratories, Chicago, IL), via right nephrectomy and ligation of two or three branches of the left renal artery, which produced infarction of approximately two-thirds of the left kidney (5/6 nephrectomy). Rats were housed under standard conditions and given unrestricted access to standard rodent chow and water. On day 2 after surgery, rats began to receive OMA (Bristol-Myers Squibb, Princeton, NJ) at 150 mg/L (n = 6) or ENA (Sigma Chemical Co., St. Louis, MO) at 100 mg/L, in the drinking water (n = 6). Sodium bicarbonate (1.5 mM) was added to solubilize OMA and was also added to the ENA solution. The doses of OMA and ENA were adjusted to achieve equivalent control of BP in the two groups; the final doses were 133 mg/L (8 to 12 mg/kg per d) for OMA and 120 mg/L (7 to 11 mg/kg per d) for ENA. Control animals received no treatment and were used for comparisons in both short- and long-term treatment protocols (n = 15). At 2-wk intervals, systolic BP (SBP) was measured using the tail-cuff method and the daily urinary protein excretion rate (Upr V) was determined by using spectrophotometry, after 3% sulfosalicylic acid precipitation of urine collected from rats individually housed in metabolic cages for 24 h. All rats were euthanized at 12 wk after surgery. The remnant kidney was perfusion-fixed with 10% phosphate-buffered formalin, delivered through a catheter in the abdominal aorta at the measured SBP for each rat.

**Micropuncture Studies**

Forty male Munich-Wistar rats underwent 5/6 nephrectomy and, at 2 to 5 d after surgery, were assigned to treatment with OMA at 25 mg/L (n = 8) or 150 mg/L (n = 8), ENA at 25 mg/L (n = 8) or 150 mg/L (n = 8), or no therapy (n = 8). Micropuncture studies were performed at 4 to 6 wk after surgery. Rats were anesthetized with Inactin (100 mg/kg body wt, intraperitoneally; Byk Gulden, Konstanz, Germany) and prepared for micropuncture as described previously (12). A PE-50 tubing catheter was placed in a femoral artery for blood sampling and monitoring of arterial BP. The euvolemic state was maintained by infusion of bovine serum albumin (4% in Ringer bicarbonate (1.5 mM) was added to solubilize OMA and was also added to the ENA solution. The doses of OMA and ENA were adjusted to achieve equivalent control of BP in the two groups; the final doses were 133 mg/L (8 to 12 mg/kg per d) for OMA and 120 mg/L (7 to 11 mg/kg per d) for ENA. Control animals received no treatment and were used for comparisons in both short- and long-term treatment protocols (n = 15). At 2-wk intervals, systolic BP (SBP) was measured using the tail-cuff method and the daily urinary protein excretion rate (Upr V) was determined by using spectrophotometry, after 3% sulfosalicylic acid precipitation of urine collected from rats individually housed in metabolic cages for 24 h. All rats were euthanized at 12 wk after surgery. The remnant kidney was perfusion-fixed with 10% phosphate-buffered formalin, delivered through a catheter in the abdominal aorta at the measured SBP for each rat.

**Long-Term Treatment Protocol**

Forty-one male Munich-Wistar rats were subjected to 5/6 nephrectomy as described above. At 4 wk after renal mass ablation, rats were assigned to two groups (matched for SBP and Upr V) and began to receive OMA (n = 22) or ENA (n = 19) in the drinking water. Doses were chosen to achieve equivalent control of SBP in the treatment groups and, after some initial adjustment, 100 mg/L OMA (6 to 9 mg/kg per d) and 100 mg/L ENA were used. SBP and Upr V were measured at 2-wk intervals. At 20 wk after surgery, some rats from each group were euthanized (20-wk set: OMA, n = 15; ENA, n = 11). These rats were identified at the beginning of treatment, at which time they were matched for SBP and Upr V. The remaining rats continued to undergo treatment until Upr V demonstrated a sustained increase to levels above pretreatment values (long-term set: OMA, n = 7; ENA, n = 8). ENA-treated rats were euthanized at 32 wk after surgery, because of rapidly increasing Upr V. For OMA-treated rats, the dose of OMA was increased to 120, 150, and 200 mg/L at 38, 39, and 44 wk after surgery, respectively, because of an increasing trend in SBP, and the rats were euthanized at 50 wk after surgery. At euthanasia, the remnant kidneys were perfusion-fixed as described above. The aforementioned study protocols were approved by the Standing Committee on Animals at Harvard Medical School.

**Morphologic Assessments**

Renal tissue was embedded in paraffin and processed for light microscopy. The frequency of glomerulosclerosis (GS) was estimated by examining all glomeruli observed in one or two coronal sections from each kidney (mean = 208 ± 9 glomeruli), which were stained by using the periodic acid-Schiff method. GS was defined as glomerular capillary collapse with hyaline deposition and/or adhesion to the parietal layer of Bowman’s capsule. GS scores were determined by expressing the number of glomeruli with segmental or global sclerosis as a percentage of the total number of glomeruli counted for each rat. Tubulointerstitial injury was assessed at medium power in the same sections. Each of three parameters of tubulointerstitial injury (tubular proteinaceous casts and dilation, interstitial inflammation, and interstitial fibrosis) was assigned a score from 0 to 3, according to severity (0, no abnormality; 1, mild; 2, moderate; 3, severe), and these scores were added to yield an overall tubulointerstitial score (TIS) of 0 to 9, which was then expressed as a percentage. The histologic assessment was performed without knowledge of the treatment assignment of individual rats.

**Statistical Analyses**

Continuous variables are expressed as mean ± SEM. Differences among multiple groups were assessed by using ANOVA and a post hoc Scheffé’s test. Parameters measured repeatedly with time were compared among groups by using repeated-measures ANOVA. A paired t test was used to compare values at different time points for the same subjects. P values of <0.05 were considered significant. Statistical analyses were conducted using Statview 4.01 (Abacus Concepts, Berkeley, CA).

**Results**

**Short-Term Treatment Protocol**

Rats from all groups gained weight in 12 wk, although the body weights of control rats tended to be moderately higher than those of OMA- or ENA-treated rats at each time point. Only the difference between control and OMA-treated rats was statistically significant. There was no difference in body...
weights between OMA-treated and ENA-treated rats with time (mean difference, 15 g; \( P = 0.3 \)). Control rats developed sustained hypertension after 5/6 nephrectomy. Treatment with either OMA or ENA prevented this increase in BP and generally maintained SBP at levels of 90 to 100 mmHg (Figure 1A). There was no significant difference in SBP with time for OMA- versus ENA-treated rats. A progressive increase in proteinuria was observed in control rats after 5/6 nephrectomy, whereas proteinuria was almost completely prevented by both treatments (Figure 1B). There was no difference in \( U_{pr}V \) between OMA- and ENA-treated rats with time. Histologic assessments of the remnant kidneys revealed substantial GS and tubulointerstitial injury in control rats, whereas histologic injury was virtually absent in OMA- and ENA-treated rats. There was no difference in GS scores or TIS for OMA- versus ENA-treated rats (Figure 1C).

**Micropuncture Studies**

The results of the micropuncture studies are presented in Table 1. Body weights were similar among the groups. Both treatments were associated with small but significant reductions in hematocrit values. Mean arterial pressures (MAP) displayed patterns similar to those of SBP in the short-term treatment protocol. Untreated rats exhibited severe hypertension, whereas ENA and OMA treatments both resulted in normalization of MAP. Although there was a trend for MAP to be lower at the higher dose of each drug, these differences were not statistically significant. As demonstrated in Table 1, all other parameters measured did not differ between the higher and lower doses of each drug; data for each treatment were therefore pooled for comparisons between treatments. Despite lower perfusion pressures, both treatments were associated with significant increases in renal plasma flow, compared with untreated control animals, in keeping with renal vasodilation. Whole-kidney GFR values, however, were not different among the groups. As previously observed, untreated rats exhibited substantial elevations in \( P_{GC} \), compared with values reported for normal rats (19). Treatment with either ENA or OMA was associated with reductions in \( P_{GC} \) to values similar to those for normal rats. At both dose levels, \( P_{GC} \) tended to be lower in OMA- versus ENA-treated rats and, when data for all OMA- and ENA-treated rats were combined, OMA treatment was associated with significantly lower \( P_{GC} \) than was ENA treatment. The glomerular transcapillary hydraulic pressure difference demonstrated similar trends, although the difference between OMA and ENA was not statistically significant. In keeping with the reductions in the glomerular transcapillary hydraulic pressure difference, the whole-kidney filtration fraction was significantly lower in both treatment groups. The single-nephron GFR and glomerular plasma flow rate were substantially higher than previously reported normal values for untreated control animals (19) and were similarly elevated in all treatment groups. Renal mass ablation was associated with substantial decreases in afferent and efferent arteriolar resistances in untreated rats, compared with previously reported normal values (19). Treatment with OMA or ENA resulted in significantly greater reductions in both afferent and efferent arteriolar resistances than the untreated group (19).

![Figure 1. Early treatment protocol. (A and B) Systolic BP (SBP) (A) and urinary protein excretion rate (\( U_{pr}V \)) (B) with time for rats that were subjected to 5/6 nephrectomy and received omapatrilat (OMA) (○), enalapril (ENA) (■), or no treatment (control) (▲) beginning on day 2 after surgery. SBP and proteinuria were normalized by both treatments and were significantly lower in treated versus untreated rats (\( P < 0.0001 \) versus control value for both SBP and proteinuria in weeks 6 to 12). (C) Glomerulosclerosis (GS) scores and tubulointerstitial injury scores (TIS) for kidneys removed at 12 wk after 5/6 nephrectomy, revealing that OMA (□) and ENA (■) effectively prevented the substantial GS and tubulointerstitial injury observed in untreated rats (control) (▲), when treatment was initiated on day 2 after surgery (*\( P < 0.005 \) versus control value).]
arteriolar resistances than did 5/6 nephrectomy alone. There were no significant differences in the glomerular capillary ultrafiltration coefficient, although values tended to be higher for the OMA- and ENA-treated groups.

Long-Term Treatment Protocol

At the initiation of treatment (4 wk), body weights were similar among the groups (\( P = 0.98 \)). Body weights increased steadily throughout the study for control rats and for rats in the 20-wk sets of both treatment groups. Among rats monitored beyond 20 wk, body weights stopped increasing after 26 wk and remained stable thereafter. No statistically significant differences in body weights between the treatment groups emerged with time, for either the 20-wk or long-term sets.

After 5/6 nephrectomy, rats in all groups developed hypertension and SBP values were similar among groups before treatment (\( P = 0.98 \)) (Figure 2). Control rats remained hypertensive until euthanasia. Treatment with either OMA or ENA decreased BP to normal levels and maintained SBP at mean levels that were generally \(<120 \text{ mmHg} \) throughout the study. No statistically significant difference in SBP between OMA- and ENA-treated rats developed with time, in either weeks 6 to 20 (mean difference, 4 mmHg; \( P = 0.3 \)) or weeks 22 to 32 (mean difference, 2 mmHg; \( P = 0.7 \)).

Rats developed substantial proteinuria during the first 4 wk after 5/6 nephrectomy, and \( U_{pr}V \) values were similar among the groups before treatment (\( P = 0.7 \)) (Figure 3). Control rats continued to exhibit a progressive increase in \( U_{pr}V \) in the subsequent 8 wk. Both OMA and ENA treatments initially reduced \( U_{pr}V \) to \(<20 \text{ mg/dL} \). However, after 12 wk there was a progressive increase in \( U_{pr}V \) among ENA-treated rats, such that mean values at 20 wk reached 81% of pretreatment values (\( P = 0.2 \) for week 20 versus week 4 values for ENA-treated rats). In contrast, \( U_{pr}V \) increased more slowly among OMA-treated rats, such that at 20 wk the mean value was only 44% of pretreatment values (\( P < 0.0001 \) for week 20 versus week 4 values for OMA-treated rats). In weeks 6 to 20, \( U_{pr}V \) values were significantly higher for ENA- versus OMA-treated rats (\( P = 0.04 \)). Beyond 20 wk, \( U_{pr}V \) continued to increase among ENA-treated rats, reaching a mean value 1.8 times that of pretreatment levels at 32 wk (\( P = 0.03 \) for week 32 versus week 4 values for ENA-treated rats). \( U_{pr}V \) increased more slowly among OMA-treated rats, such that 50-wk levels were similar (1.2 times) to pretreatment values (\( P = 0.3 \) for week 50.
Table 1. (Cont’d)

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<th>(P_e) (mmHg)</th>
<th>(\Delta P) (mmHg)</th>
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<th>(Q_A) (nl/min)</th>
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<th>(R_E \times 10^{10}) (dyne (\cdot s) (\cdot cm^{-5}))</th>
<th>(K_r) [nl/(s \cdot mmHg)]</th>
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<td>18 ± 1(^b)</td>
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<td>56 ± 6</td>
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<td>0.74 ± 0.10(^c)</td>
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\(^a\) Hct, hematocrit; MAP, mean arterial pressure; RPF, renal plasma flow; FF, filtration fraction; \(C_A\), afferent arteriolar protein concentration; \(P_{SC}\), glomerular capillary hydraulic pressure; \(P_f\), proximal tubule hydraulic pressure; \(P_E\), efferent arteriolar hydraulic pressure; \(\Delta P\), glomerular transcapillary hydraulic pressure difference; SNGFR, single-nephron GFR; \(Q_A\), glomerular plasma flow rate; \(R_A\), afferent arteriolar resistance; \(R_E\), efferent arteriolar resistance; \(K_r\), glomerular capillary ultrafiltration coefficient.

\(^b\) \(P < 0.05\) versus control value.

\(^c\) \(P < 0.05\) for combined 25 and 150 mg/L ENA data or combined 25 and 150 mg/L OMA data versus control value.

\(^d\) \(P < 0.05\) for combined 25 and 150 mg/L OMA data versus combined 25 and 150 mg/L ENA data.

versus week 4 values). In weeks 22 to 32, \(U_{pr}\) \(V\) values were significantly higher for ENA- versus OMA-treated rats (\(P = 0.03\)). In the long-term sets, six of eight rats in the ENA-treated group and five of seven rats in the OMA-treated group exhibited \(U_{pr}\) \(V\) levels greater than pretreatment values at the time of euthanasia. However, the mean time to reach a \(U_{pr}\) \(V\) level greater than the pretreatment level in two readings was significantly shorter for ENA- versus OMA-treated rats (20 wk versus 34 wk, respectively; \(P = 0.002\)).

Histologic assessment of remnant kidneys revealed a mean GS score of 36.1% for control rats at 12 wk after 5/6 nephrectomy (Figure 4). For OMA-treated rats at 20 wk, the GS score was significantly lower than this value (GS score, 13.9%; \(P = 0.001\) versus control). A similar trend was observed for ENA-treated rats at 20 wk, although the difference was not statistically significant (GS score, 22.1%; \(P = 0.3\) versus control). The GS score tended to be lower for OMA-treated rats, compared with ENA-treated rats, at 20 wk, but this difference also was nonsignificant (\(P = 0.8\)). In the long-term set, ENA-treated rats euthanized at 32 wk (GS score, 34 ± 5%) and OMA-treated rats euthanized at 50 wk (GS score, 38 ± 8%) exhibited mean GS scores very similar to those of untreated control rats at 12 wk. Tubulointerstitial injury exhibited a pattern similar to that of GS. At 20 wk, the TIS was significantly lower for OMA-treated versus control rats and tended to be lower for ENA-treated versus control rats. In the long-term set, OMA-treated rats at 50 wk exhibited a mean TIS similar to that of control rats at 12 wk. The TIS for ENA-treated rats at 32 wk tended to be lower than that for OMA-treated rats at 50 wk, but this difference was not statistically significant (Figure 4). Overall, there was a highly significant correlation between GS scores and TIS for individual rats (\(r = 0.82, P < 0.0001\)).

**Discussion**

**Short-Term Treatment Protocol**

We demonstrated that, when treatment was initiated early after 5/6 nephrectomy, at doses sufficient to normalize BP, both OMA and ENA almost completely prevented the progressive increase in proteinuria and histologic renal injury observed in untreated control animals in 12 wk. Because ENA treatment was previously demonstrated to result in almost complete renoprotection in this model (12,20), we did not expect any greater benefit with OMA than ENA. Other studies comparing VPI and ACEI treatment in early treatment protocols after 5/6 nephrectomy reported greater renoprotective effects with the VPI (13,14). In one of those studies, however, BP was substantially different between the treatment groups at the lower dose studied and was not normalized in either group, even at a higher dose (13). In the other study, SBP was also significantly higher in ACEI-treated than VPI-treated rats (14). These previous data are therefore unable to distinguish additional renoprotective effects attributable to greater systemic BP reduction from effects related to the unique renal actions of VPI.

**Micropuncture Studies**

Previous micropuncture studies in the partial-nephrectomy model demonstrated that elevated \(P_{GC}\) is a critical factor in the pathogenesis of progressive renal injury, and the renoprotective effects of low-protein diet, ACEI, or angiotensin subtype 1 receptor antagonist treatment were closely associated with normalization of \(P_{GC}\) (12,19,21,22). In this study, ENA and OMA produced similar effects on most of the hemodynamic parameters studied. Importantly, MAP was decreased to the same extent with both treatments. The observed increases in renal plasma flow, together with a reduction in the filtration fraction, suggest renal vasodilation and a reduction of \(P_{GC}\). Measurements of single-nephron hemodynamics confirmed that both afferent and efferent arteriolar resistances were reduced and \(P_{GC}\) was significantly decreased by both treatments. The \(P_{GC}\) values observed for ENA-treated rats were very similar to those previously reported for ENA-treated rats (12,21,22). In contrast, OMA treatment was associated with greater reductions of \(P_{GC}\) than was ENA treatment. As expected, \(P_{GC}\) was significantly correlated with MAP when data for all rats were pooled (\(r = 0.69, P < 0.0001\)). As shown in Figure 5, however, regression lines for \(P_{GC}\) versus MAP for the treatment groups demonstrated that, at each level of MAP, OMA decreased \(P_{GC}\) to a greater extent than did ENA. We
suggest that this greater lowering of $P_{GC}$ at equivalent levels of MAP represents one of the mechanisms whereby OMA produces greater renoprotection. The mechanism whereby greater lowering of $P_{GC}$ was achieved with OMA is not revealed by our data, because the two treatments had equivalent effects on afferent and efferent arteriolar resistances. There was, however, a trend toward a greater afferent arteriolar resistance in OMA-treated rats, such that levels at week 20 remained significantly lower than pretreatment values and $U_{p}V$ values at week 32 were similar to pretreatment values. $U_{p}V$ values were significantly lower for OMA-versus ENA-treated rats in weeks 6 to 20 and in weeks 22 to 32. At week 32, the mean $U_{p}V$ for ENA-treated rats was more than twice that for OMA-treated rats. *$P < 0.05$ versus control value in weeks 6 to 12; ‡$P < 0.05$ versus pretreatment value; †$P < 0.05$ versus ENA in weeks 6 to 20; §$P < 0.05$ versus ENA in weeks 22 to 32.

Figure 3. Delayed treatment protocol. $U_{p}V$ was measured with time for rats that were subjected to 5/6 nephrectomy and treated with OMA (○), ENA (□), or no treatment (control) (▲) beginning 4 wk after surgery. Rats from all groups developed substantial proteinuria before treatment and were closely matched for $U_{p}V$ at 4 wk after surgery. Treatment with either OMA or ENA resulted in an initial reduction in proteinuria, such that $U_{p}V$ values for OMA- and ENA-treated rats were significantly lower than those for untreated rats for weeks 6 to 12 ($P < 0.0001$). $U_{p}V$ increased after week 10 among ENA-treated rats, such that levels at week 20 were no longer significantly lower than pretreatment values and $U_{p}V$ values at week 32 were significantly higher than pretreatment values. $U_{p}V$ increased more slowly among OMA-treated rats, such that levels at week 20 remained significantly lower than pretreatment values and levels at week 50 were similar to pretreatment values. $U_{p}V$ values were significantly lower for OMA-versus ENA-treated rats in weeks 6 to 20 and in weeks 22 to 32. At week 32, the mean $U_{p}V$ for ENA-treated rats was more than twice that for OMA-treated rats. *$P < 0.05$ versus control value in weeks 6 to 12; ‡$P < 0.05$ versus pretreatment value; †$P < 0.05$ versus ENA in weeks 6 to 20; §$P < 0.05$ versus ENA in weeks 22 to 32.

Figure 4. Delayed treatment protocol. GS scores and TIS were measured for rats that were subjected to 5/6 nephrectomy and treated with OMA (○), ENA (□), or no treatment (control) (■) beginning 4 wk after surgery. Both treatments resulted in protection from histologic injury, such that GS scores and TIS for OMA- and ENA-treated rats at week 20 were lower than those for control rats at week 12, although only the difference between OMA-treated and control rats was statistically significant. GS scores for control rats at week 12, ENA-treated rats at week 32, and OMA-treated rats at week 50 were similar, suggesting that ENA delayed the progression of glomerular injury by 20 wk, whereas OMA delayed it by 38 wk. TIS demonstrated a similar trend, although ENA-treated rats at week 32 tended to exhibit less tubulointerstitial injury. *$P < 0.005$ versus control value.

suggest that additional renoprotection may be achieved with OMA in longer-term studies.

Long-Term Treatment Protocol

We previously observed that, in 5/6 nephrectomized rats, when treatment with an ACEI or angiotensin subtype 1 receptor antagonist was delayed until after the onset of renal injury, an initial decrease in proteinuria was followed by a slow but progressive increase (23). This model of slow progression of CRD during inhibition of the renin-angiotensin system more
closely resembles the findings of clinical trials that have reported slowing rather than arrest of CRD progression with ACEI treatment (2–5). We therefore think that this is an appropriate model in which to investigate the possibility that VPI may offer clinically relevant renoprotective advantages, compared with ACEI alone. We observed that, although both treatments initially decreased proteinuria to the same extent, OMA maintained significantly lower levels of proteinuria with time than did ENA. Moreover, the mean time for rats to reach pretreatment levels of proteinuria was prolonged by 14 wk for OMA- versus ENA-treated rats (34 wk versus 20 wk). Although this study was not designed for assessment of survival rates, the fact that ENA-treated rats were euthanized at 32 wk because of rapidly increasing proteinuria, whereas OMA-treated rats survived to 50 wk, indicates that OMA-treated rats would have survived longer than ENA-treated rats. The histologic findings also attest to more effective renoprotection with OMA. Among rats euthanized at 20 wk after 5/6 nephrectomy, both treatment groups exhibited GS scores and TIS that tended to be lower than those of untreated control animals at 12 wk, indicating that both treatments delayed the progression of renal injury. Injury scores tended to be lower for OMA-treated rats, compared with ENA-treated rats, suggesting a possible advantage of OMA over ENA at this relatively early time point. ENA-treated rats euthanized at 32 wk exhibited GS scores very similar to those of untreated control rats at 12 wk, indicating that ENA delayed the progression of CRD by 20 wk. GS scores for OMA-treated rats euthanized at 50 wk were also similar to 12-wk control values, indicating a 38-wk delay in progression to this level. Therefore, long-term treatment with OMA resulted in almost twice the delay in CRD progression achieved with ENA. The dose of ENA used in this study was the same as or higher than that used to achieve renoprotection in previous studies with this model (23–25), indicating that the observed differences between ENA and OMA treatments were not attributable to inadequate ENA dosing. Finally, it should be noted that these differences were observed between groups of rats in which BP values were very closely matched. We are thus able to conclude that the additional benefit observed with OMA was attributable to unique renal actions of VPI and not to differences in systemic BP control. The micropuncture studies discussed above suggest that one of these differences may be more effective lowering of $P_{GC}$ with OMA. Unfortunately, the micropuncture studies required to support this hypothesis are complicated by the heterogeneity of the glomerular lesions typical of this model, making it impossible to obtain measurements from the few available surface glomeruli that are representative of the remnant kidney as a whole.

Further studies will be required to elucidate the specific roles of different biochemical mediators, particularly the natriuretic peptides and bradykinin, in the enhanced renoprotective efficacy observed with OMA. The natriuretic peptides are known to mediate some of the hemodynamic changes after 5/6 nephrectomy (26) but also exert nonhemodynamic effects that may contribute to renoprotection (27–31). Bradykinin is a potent vasodilator substance that may mediate some of the antihypertensive effects of ACEI (32) but does not seem to contribute significantly to the renoprotective effects of ACEI in rats after 5/6 nephrectomy (33,34) or induction of diabetes mellitus (35). Nevertheless, in other models, bradykinin may mediate effects that are potentially beneficial in renoprotection (36–39). Moreover, renal bradykinin levels are likely to be higher with VPI treatment than with ACEI treatment, because bradykinin is catabolized by both ACE and NEP (40) and NEP is the major route of bradykinin catabolism in the kidney (41). The effects of NEP inhibition on other vasoactive molecules, such as endothelin-I, may also be important (42). It is of course possible that the additional renoprotective efficacy of OMA results from a combination of all of the aforementioned effects and other, currently unidentified effects.

**Conclusion**

We have demonstrated that, despite equivalent control of systemic BP, the VPI OMA almost doubles the delay in CRD progression achieved with the ACEI ENA in rats subjected to 5/6 nephrectomy and prolonged follow-up monitoring. If clinical studies produce similar results, then this new class of drugs may have a major effect in reducing the number of patients with CRD that progresses to end-stage renal failure. We suggest that greater lowering of $P_{GC}$ may be one of the mechanisms whereby OMA treatment results in more effective renoprotection, but further studies are required to fully elucidate the mechanisms involved.

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Figure 5. Micropuncture. Means for the plot of glomerular capillary hydraulic pressure ($P_{GC}$) versus mean arterial pressure (MAP) for rats that were subjected to 5/6 nephrectomy and treated with OMA (○), ENA (■), or no treatment (control) (▲) beginning days 2 to 5 after surgery are presented. Values were obtained during micropuncture studies at 4 to 6 wk after 5/6 nephrectomy. The dashed and solid lines represent least squares linear regression slopes for $P_{GC}$ versus MAP for ENA- and OMA-treated rats, respectively.

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