

Hemodynamic and Humoral Effects of Vasopeptidase Inhibition in Canine Hypertension

Calin V. Maniu, Donna M. Meyer, Margaret M. Redfield

Abstract—Vasopeptidase inhibitors are potent new antihypertensive agents. The dual inhibition of ACE and neutral endopeptidase may result in synergistic humoral effects with unique hemodynamic actions. We investigated the hemodynamic and neurohumoral effects of vasopeptidase inhibition in conscious dogs made hypertensive by bilateral renal wrapping and subsequently instrumented for long-term assessment of left ventricular pressure and volume ($n=8$). Intravenous vasopeptidase inhibition (omapatrilat, $30 \mu\text{mol/kg}$ over 10 minutes) reduced peak left ventricular pressure (171 ± 6 versus 130 ± 6 mm Hg immediately after infusion, $P<0.01$) through arterial vasodilation (arterial elastance, 9.8 ± 0.8 to 5.8 ± 1.6 mm Hg/mL, $P<0.01$) and preload reduction (left ventricular end-diastolic volume, 51.1 ± 6.8 to 46.0 ± 6.9 mL, $P<0.01$). At 60 minutes, preload decreased further (40.5 ± 5.9 mL, $P<0.01$ versus baseline). Vasopeptidase inhibition increased plasma levels of adrenomedullin (41.2 ± 9.6 versus 72.3 ± 15 pg/mL, $P<0.01$), whereas levels of the natriuretic peptides and cGMP were unchanged. Similar hemodynamic and humoral effects were observed with long-term therapy. Neither an equimolar dose of an ACE inhibitor (fosinopril) nor exogenous adrenomedullin had as potent of a hypotensive effect, and neither reduced preload. In summary, the potent short-term and long-term hypotensive effects of vasopeptidase inhibition were prominently mediated by preload reduction, an effect not reproduced by ACE inhibition nor adrenomedullin augmentation and not associated with enhanced natriuretic peptide levels. Combined arterial vasodilation and preload reduction may confer additional potency as well as unique cardioprotective effects. Synergistic effects on humoral and probably endothelial vasodilatory factors appear to be important in mediating the unique hemodynamic profile of vasopeptidase inhibition in this form of experimental hypertension. (*Hypertension*. 2002;40:528-534.)

Key Words: hypertension, experimental ■ hemodynamics ■ natriuretic peptides ■ angiotensin ■ drug therapy

Hypertension is a highly prevalent condition proven to lead to vascular disease, renal insufficiency, and congestive heart failure. Less than 30% of patients with hypertension are adequately controlled, and more effective treatments are needed. Optimal treatment of hypertension must be coupled to an understanding of the hemodynamic, humoral, and renal effects of pharmacological agents.

Vasopeptidase inhibitors (VPI) are novel compounds^{1,2} that provide dual inhibition of ACE and neutral endopeptidase (NEP). Thus, VPI block angiotensin II (Ang II) production and inhibit degradation of bradykinin,³ natriuretic peptides, adrenomedullin (ADM),⁴ and substance P.² VPI may provide a new approach to antihypertensive therapy whereby vasoconstrictor systems are antagonized and a number of vasodilatory factors are enhanced. Although preliminary studies indicate that VPI are more potent in more types of hypertension than ACE inhibitors (ACE-I) alone,^{2,5} the neurohumoral effects of VPI in hypertension and the hemodynamic mechanisms (preload reduction versus arterial dilatation) whereby VPI exert their antihypertensive effects have not been well described.

In the current study, we used a canine model of renal hypertension, chronically instrumented for conscious assessment of ventricular volume and pressure, to define the humoral and hemodynamic mechanisms whereby short-term VPI administration exerts antihypertensive actions. We compared the effects of VPI with those of ACE-I to define the contribution of ACE inhibition to the hypotensive effects of VPI. We defined the effects of VPI on circulating levels of vasodilatory peptides and assessed the contribution of vasodilatory peptide activation to the hypotensive effects of VPI. Because short-term and long-term effects of humoral modulation may differ, we also examined the long-term effects of VPI therapy on hemodynamics and humoral function.

Methods

Experiments were performed in male mongrel dogs (weight, 20 to 28 kg) maintained on standard sodium diet (0.42%) with free access to water. All experimental procedures were approved by the Mayo Institutional Animal Care and Use Committee.

Model of Hypertension

Dogs ($n=10$) were made hypertensive by the "Page model" of bilateral renal wrapping, as previously characterized in our labora-

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From the Division of Cardiovascular Diseases, Mayo Clinic and Mayo Foundation, Rochester, Minn.

Correspondence to Dr Margaret M. Redfield, Mayo Clinic and Foundation, Cardiorenal Laboratory, Guggenheim 9, 200 First St, SW, Rochester, MN 55905. E-mail redfield.margaret@mayo.edu

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tory.^{6,7} An indwelling femoral arterial catheter was placed for monitoring of blood pressure (BP).

Cardiac Instrumentation

Five to 7 weeks after renal wrapping, dogs underwent instrumentation of the left ventricle (LV) with a micromanometer pressure transducer, a silicon fluid-filled catheter, and piezoelectric dimension crystals for assessment of LV volume, as previously described.^{8,9}

Experimental Protocols

Short-term studies were performed 2 to 3 weeks after cardiac instrumentation in 8 conscious standing dogs.

Short-term Effects of VPI Versus ACE-I

Dogs underwent 2 short-term studies on separate days. After baseline recordings, VPI (omapatrilat, 30 μ mol/kg IV over 10 minutes) or ACE-I (fosinopril, 30 μ mol/kg IV over 10 minutes) was administered. Hemodynamic data were recorded immediately, 30 minutes, and 60 minutes after the study drug infusion. Venous blood samples were collected at baseline and at 30 and 60 minutes. The dose of omapatrilat used had previously been demonstrated to have a hypotensive effect in the conscious dog (data on file, Bristol-Myers Squibb). To compare the hemodynamic effects of VPI to ACE-I, we used an equimolar dose of the ACE-I. Administration of equimolar doses of these 2 drugs had previously been demonstrated to produce similar inhibition of ACE.¹ Finally, we confirmed the ACE inhibitory effects of the doses used by performing the Ang I (310 ng/kg IV) pressor test before and after administration of each drug in a series of 4 experiments with each drug performed, on separate days, in 2 normal dogs instrumented with an indwelling arterial catheter.

Adrenomedullin Infusion

Each hypertensive dog underwent a third experiment involving the administration of human ADM at a dose of 20 ng/kg per minute IV for 30 minutes, followed by 100 ng/kg per minute IV for 30 minutes. Hemodynamic data and venous blood samples were obtained at baseline and at the end of each 30-minute infusion.

Long-Term VPI Therapy

After completion of the last short-term study, 5 dogs were started on oral omapatrilat therapy at a dose of 20 mg/kg twice daily. After 1 week of therapy, hemodynamic and humoral data were obtained 2 to 4 hours after the last oral dose of VPI.

Dogs were euthanized by anesthetic overdose, consistent with guidelines of the Panel on Euthanasia of the American Veterinary Medical Association.

Hemodynamic Analysis

Pressure and dimension signals were acquired and analyzed with CardioSoft (Sonometrics Corporation), as previously described.⁹

Humoral Analysis

Radioimmunoassays for ADM, canine brain natriuretic peptide (cBNP), atrial natriuretic peptide (ANP), C-type natriuretic peptide (CNP), plasma renin activity (PRA), aldosterone, cAMP, and cGMP were performed as previously described.⁷⁻⁹ Catecholamines were measured by high-performance liquid chromatography.

Statistical Analysis

Data were averaged and reported as mean \pm SEM. Comparison of the data were performed with the 2-tailed Student's *t* test for paired data, with the use of Bonferroni's correction for multiple comparisons, as indicated. A probability value <0.05 was considered significant for simple paired *t* tests, and a probability value <0.05 divided by the number of comparisons was considered significant for multiple comparisons. Since the variability of neurohormones increased with their mean level, a log transformation of the data was used in some analyses.

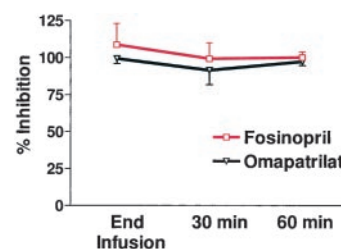


Figure 1. Inhibition of Ang I pressor response by omapatrilat and fosinopril infusion.

An expanded Methods section can be found in an online supplement. Please see <http://hyper.ahajournals.org>.

Results

Model of Systemic Hypertension

Mean arterial pressure was 151 ± 8 mm Hg at 2 weeks and 179 ± 11 mm Hg at 4 weeks after renal wrapping. This is elevated as compared with normal dogs and consistent with our previous experience in this model.⁷ Compared with that observed before renal wrapping, the neurohumoral profile obtained after wrapping and cardiac instrumentation and immediately before the short-term studies revealed significant increases in PRA (9.0 ± 1.1 versus 4.6 ± 1.5 ng/mL per hour, $P=0.03$), aldosterone (67 ± 14 versus 2 ± 1 ng/dL, $P=0.001$), cBNP (397 ± 129 versus 31 ± 7 pg/mL, $P=0.02$), and ADM (73 ± 25 versus 14 ± 2 pg/mL, $P=0.002$). There was no significant change in the ANP or CNP levels.

ACE-Inhibitory Effect of VPI and ACE-I

The doses of omapatrilat and fosinopril both provided essentially complete inhibition of the pressor response to Ang I stimulation (Figure 1).

Hemodynamic Effects of Short-Term VPI Versus ACE-I

Short-term VPI administration resulted in significant reductions in LV peak and end-systolic pressures that were evident at end infusion and sustained at 30 and 60 minutes (Figure 2). Short-term VPI also resulted in prompt and progressive reduction in preload with decreases in end-diastolic LV pressure and volume. Preload reduction was associated with a reduction in stroke volume at 30 and 60 minutes. Arterial elastance (E_a), a parameter that reflects the compliance, characteristic impedance, and resistive properties of the vasculature, was significantly decreased at end infusion but returned toward normal at 30 and 60 minutes despite the continued hypotensive effect. Compared with baseline, there was a trend toward an increase in heart rate with VPI (137 ± 8 bpm at baseline, 161 ± 15 bpm, $P=0.06$ at end infusion, 154 ± 11 bpm, $P=0.10$ at 30 minutes and 164 ± 9 bpm, $P=0.10$ at 60 minutes). In contrast to VPI, short-term ACE-I had no significant hemodynamic effect over the 60-minute observation period.

Neurohumoral Effects of Short-Term VPI Versus ACE-I

Neither VPI nor ACE-I produced significant changes in PRA or aldosterone levels (data not shown). Despite reduction in

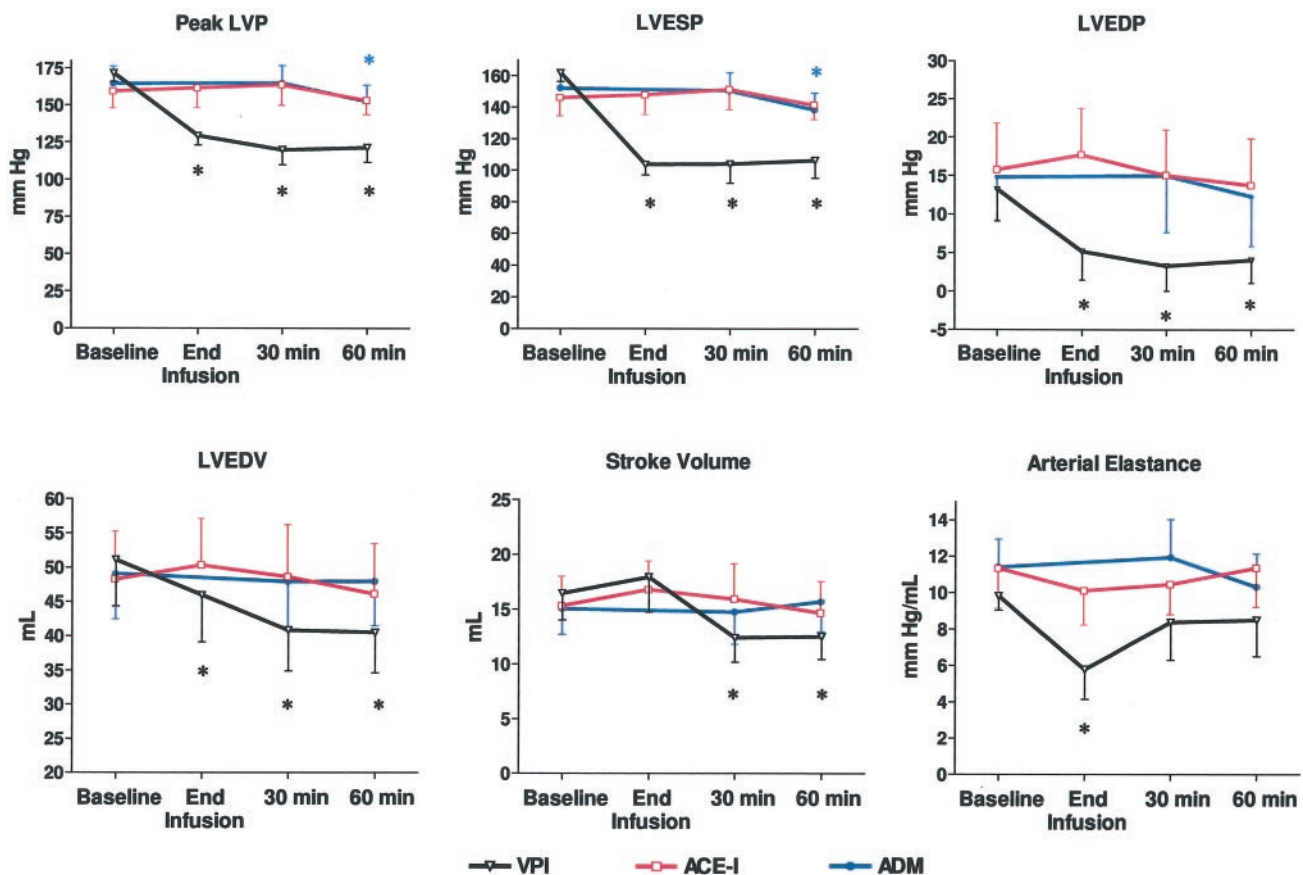


Figure 2. Hemodynamic effects of short-term VPI, ACE-I, and ADM infusion. LVP indicates left ventricular pressure; LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume. * $P < 0.01$ vs baseline.

filling pressures, there was no significant change in ANP, cBNP, or CNP or their second messenger cGMP with VPI, though cBNP tended to decrease ($P = 0.09$) at 60 minutes (Figure 3). The plasma levels of ADM and norepinephrine increased significantly with VPI. Although there was a trend toward a decrease in ANP at 30 minutes after ACE-I, ACE-I had no other significant effects on the neurohumoral parameters.

Hemodynamic and Neurohumoral Effects of ADM Infusion

Because ADM was the only vasodilatory peptide whose concentration was augmented by VPI therapy, we examined the hemodynamic significance of increased ADM levels by administering ADM at doses designed to mimic the levels produced by VPI. The low-dose ADM infusion approximated the levels of ADM achieved with VPI (49 ± 9 pg/mL baseline to 112 ± 32 pg/mL, $P = 0.007$) but did not influence LV pressure (Figure 2). The higher dose produced higher ADM levels (304 ± 166 pg/mL, $P = 0.06$ versus baseline) and was associated with a modest but significant decrease in peak and end-systolic LV pressure. There were no significant changes in end-diastolic LV pressure or volume or stroke volume with either dose. However, heart rate increased progressively with ADM (134 ± 8 bpm baseline versus 154 ± 8 bpm at 60 minutes, $P = 0.004$) and resulted in a trend toward an increase

in cardiac output at 60 minutes (2.00 ± 0.32 L/min at baseline versus 2.47 ± 0.45 L/min at 60 minutes, $P = 0.027$). E_a tended to be lower at 60 minutes. There were no changes in PRA, aldosterone, catecholamines, cGMP, or cAMP levels with the ADM infusion (data not shown).

Hemodynamic and Neurohumoral Effects of Long-Term VPI Therapy

The short-term hemodynamic and humoral effects of VPI were maintained with long-term (1 week) therapy (Figure 4). The long-term reduction in LV peak systolic pressure and end-diastolic volumes were similar to those seen at 60 minutes after short-term infusion. There was no significant change in E_a with long-term therapy. Figure 5 illustrates the effect of short-term and long-term VPI therapy on the LV pressure-volume loop in a single dog. The increases in plasma levels of ADM were maintained with long-term therapy. There was a modest increase in CNP with long-term therapy. Despite long-term reduction in preload, plasma levels of ANP and cBNP were not significantly changed, although there were trends toward decreases in both. There was no change in cGMP or PRA with long-term therapy, although aldosterone tended to decrease (data not shown).

Discussion

We have demonstrated the potent antihypertensive effect of short-term and long-term VPI administration in experimental

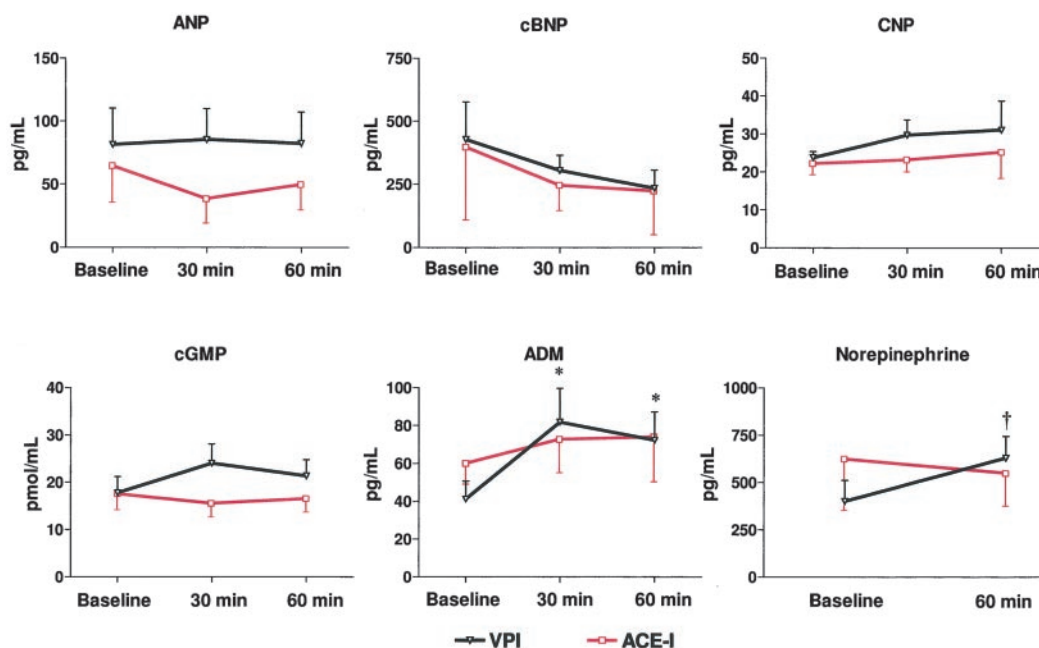


Figure 3. Neurohumoral effects of short-term VPI and ACE-I infusion. * $P < 0.01$ vs baseline; † $P < 0.05$ vs baseline.

renal hypertension and defined the hemodynamic mechanisms that mediate the hypotensive effect. Furthermore, we provided insight into the neurohumoral mechanisms mediating the observed hemodynamic effects. We found evidence of short-term arterial vasodilation along with potent and progressive preload reduction. Although VPI has been demonstrated to reduce LV volume in dogs with experimental congestive heart failure,¹⁰ such an effect has not been examined in hypertension. The dose of VPI used provided potent ACE inhibition. However, this alone did not appear to mediate the hypotensive effect because administration of an equimolar dose of an ACE-I had minimal short-term hemodynamic effect. Associated with the marked preload reduction, levels of ANP and cBNP tended to decrease. Plasma ADM levels were consistently increased with short-term and long-term VPI therapy. However, the administration of exogenous ADM to mimic or exceed the levels achieved with VPI therapy did not reproduce the dramatic reductions in LV pressure or volume seen with VPI administration. The current data in a large animal model of hypertension underscore the unique hemodynamic and complex neurohumoral effects of VPI administration in hypertension.

The use of this large animal model of hypertension affords the opportunity to simultaneously examine the hemodynamic and neurohumoral mechanisms involved in the hypotensive effects of VPI. The mechanism of "Page hypertension" was originally thought to be related to activation of the renin-angiotensin system.^{6,11} In a previous study, we found no changes in PRA or Ang II or endothelin levels in the plasma, left ventricle, or kidneys.⁷ Other studies that used several variations of the "Page model" yielded conflicting results as to the role of the renin-angiotensin system activation in this model of hypertension as well as to the effect of antagonism of the renin-angiotensin system in the model. In contrast to our previous experience in a large number of animals, we saw

increases in PRA and aldosterone in hypertensive dogs. However, we did not assess the humoral profile after renal wrapping but before cardiac instrumentation, and we speculate that the increases in PRA and aldosterone may be linked to the considerable stress of the second surgery in animals with rapidly developing severe hypertension. Furthermore, the minimal effect of short-term ACE-I suggests that this model is not highly renin-dependent. A novel observation in the current study is the increased ADM level observed in the hypertensive dogs. The ADM values before renal wrapping (14 pg/mL) were similar to the ones previously reported by our laboratory in normal, chronically instrumented dogs (9 pg/mL).⁹ In normotensive animals, ADM is a potent vasodilator that may function as a hormonal or autocrine/paracrine factor.¹² The elevated ADM levels observed in the model could represent a compensatory mechanism to lower BP. Although further studies are needed to better understand the mechanism of "Page hypertension," more than 100 studies have used this model to evaluate the physiology and pharmacology of hypertension.

VPI provide a novel form of neurohumoral modulation, which could offer unique benefit in the treatment of hypertension. Although animal studies report that VPI effectively lower BP in different models of experimental hypertension,^{13–15} regardless of the renin level or volume status,¹⁶ their relative potency compared with ACE-I may vary according to the mechanism of hypertension. Studies in diabetic spontaneously hypertensive rats show greater reduction in BP with VPI as compared with ACE-I alone, whereas the response is similar in nondiabetic spontaneously hypertensive rats.¹⁷ In salt-sensitive hypertensive patients, VPI was more effective than ACE-I.¹⁸ In the current study, the objective was not to compare the potency of VPI and ACE-I per se but to investigate the individual components of the humoral response in the context of the hemodynamic effects seen.

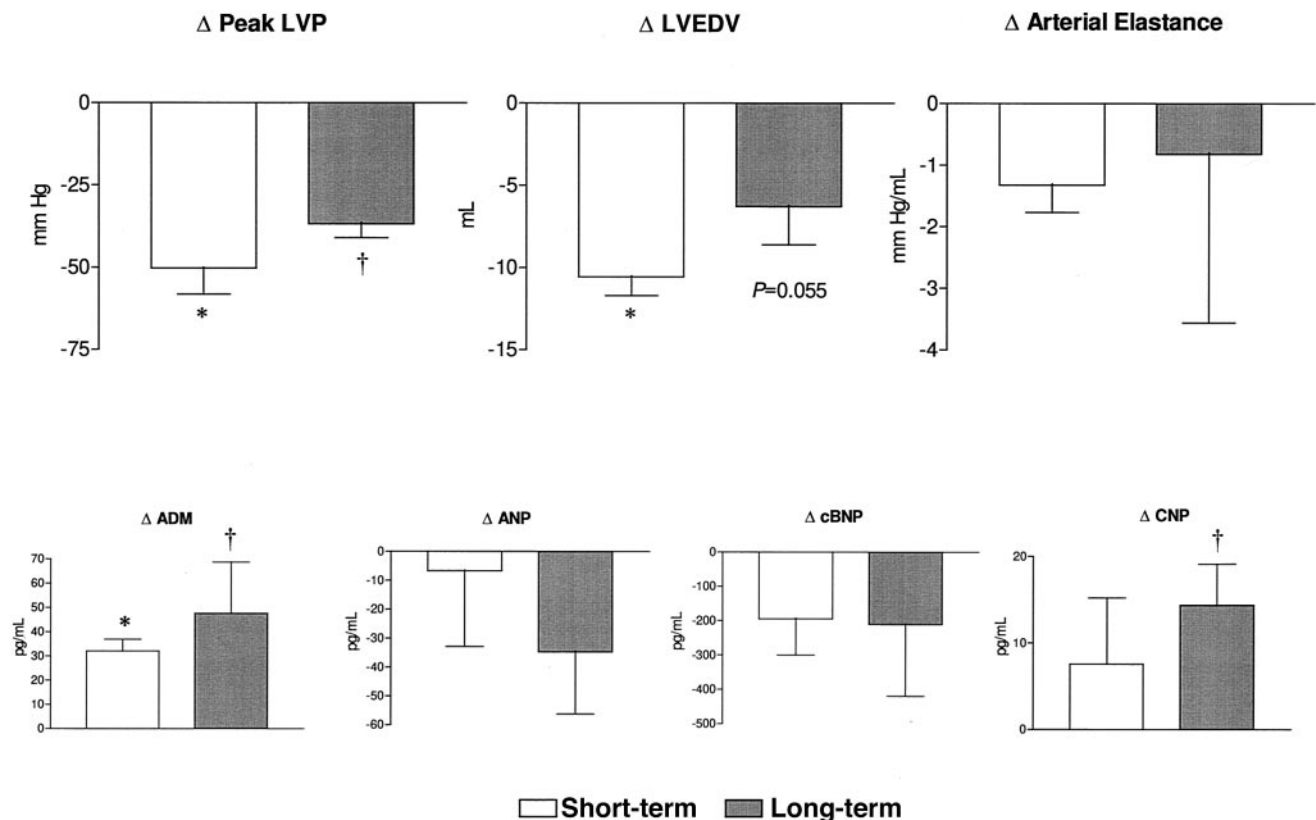


Figure 4. Hemodynamic and humoral effects of short-term (n=8) and long-term (n=5) VPI administration. * $P < 0.01$ vs baseline; † $P < 0.05$ vs baseline before starting oral omapatrilat. LVP indicates left ventricular pressure; LVEDV, left ventricular end-diastolic volume.

Preload reduction and pure arterial vasodilation can reduce BP, and each will influence the other. In the current study, we found evidence of rapid arterial vasodilation with VPI, as E_a was significantly reduced immediately after infusion and was associated with trends toward increases in stroke volume and cardiac output. However, as preload continued to decrease, arterial tone returned toward baseline despite continued hypotension, probably representing reflex vasoconstriction in response to the dramatic preload reduction. The effects on preload were maintained with long-term administration and suggest that preload reduction is an important component of

VPI antihypertensive effects. The combination of reduction in preload along with arterial vasodilation may contribute to the more potent antihypertensive effects of VPI when compared with other agents.^{2,5}

The effects of VPI therapy on humoral function were examined in an effort to gain insight into the mechanisms whereby VPI reduces preload and afterload in renal hypertension. Both VPI and ACE-I demonstrated potent ACE inhibition, as evidenced by suppression of the hypertensive response to Ang I administration, although PRA did not rise acutely with either agent, as has been noted in some previous studies.^{19–21} In human hypertension, ACE-I reduce BP through effects on arterial tone. As Ang II has little or no direct effect on venous smooth muscle,²² any venodilation present may be mediated by withdrawal of venous sympathetic tone and/or removal of the synergistic effect of Ang II on peripheral sympathetic activity.²³ The rapid and dramatic effect on preload and arterial tone seen with short-term VPI but not short-term ACE-I suggests that other mechanisms contribute to the hemodynamic effect in this form of hypertension.

Systemic natriuretic peptide administration in experimental and human heart failure and isolated forearm administration in normal humans reduces arterial resistance.^{24,25} Pharmacological doses of natriuretic peptides produce modest reduction in end-diastolic volume in the normal dog and in experimental canine heart failure.⁸ Short-term VPI administration in experimental heart failure increases natriuretic

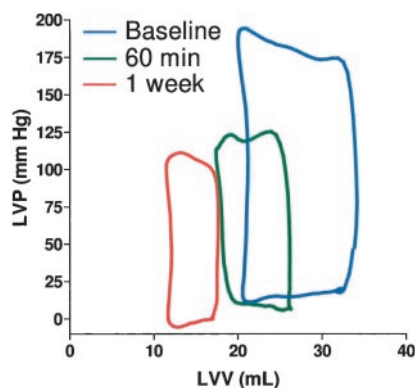


Figure 5. Hemodynamic effects of short-term and long-term VPI administration in a single dog. LVP, Left ventricular pressure; LVV, left ventricular volume.

peptide levels, an effect that contributes to the renal and humoral actions of VPI.²⁶ Thus, we postulated that augmentation of natriuretic peptides might contribute to the antihypertensive effects of VPI. However, effects on natriuretic peptide levels were minimal with modest augmentation of CNP chronically and a tendency for ANP and cBNP to decrease in association with reduction in filling pressures. There was no evidence of enhanced natriuretic peptide activity, as cGMP levels were not increased. Previous studies in experimental hypertension have also reported no change in natriuretic peptide levels in response to long-term VPI.¹³ Thus, in contrast to heart failure, in which the activation of natriuretic peptides is more dramatic, we find no augmentation of plasma natriuretic peptide levels with inhibition of their degradation in the face of dramatic preload reduction. It is well established that increased cardiac preload is a major stimulus for ANP and BNP release, and a relative maintenance of natriuretic peptide levels in the face of cardiac unloading may contribute to the synergistic humoral actions of VPI. Furthermore, whereas changes in the renal levels of natriuretic peptides were not assessed, these might play an important role in the complex actions of VPI. In a study of salt-sensitive hypertensive subjects, VPI was associated with increased urinary excretion of ANP and cGMP, whereas ACE-I had no effect.¹⁸

There was a consistent and significant increase in ADM with short-term and long-term VPI therapy. There are inconsistent data regarding the response of plasma ADM levels when hypertension is treated.^{27,28} Consistent with our findings in hypertension, increases in ADM have been noted with VPI therapy in heart failure, an expected effect in the presence of NEP inhibition.²¹ However, a previous study in a salt-sensitive rat model of hypertension reported reduction in plasma ADM levels with VPI treatment.¹⁵ ADM infusion reduces BP in humans with hypertension²⁹ and in normal dogs, in which it acts as an arterial vasodilator without effect on preload.⁹ In the current study, infusion of ADM resulted in a hypotensive effect only, with plasma levels that exceeded those observed with VPI. This effect was unassociated with reduction in preload and was far less dramatic than that observed with VPI. Although ADM probably contributed to the arterial vasodilation observed, it has not been shown to reduce preload when given alone, even at pharmacological doses associated with a marked hypotensive effect.⁹

Dual NEP/ACE inhibition may produce far more potent bradykinin augmentation with stimulation of a number of vasodilatory substances (nitric oxide, endothelium-derived hyperpolarizing factor, prostacyclin), all of which may produce reductions in arterial tone and preload. Although the rapid onset of the hypotensive effect suggests involvement of the nitric oxide pathway, we must acknowledge preliminary reports in bradykinin receptor knock-out mice that suggest that bradykinin does not mediate the superior hypotensive effect of VPI over ACE-I.³⁰ The effect of VPI on endothelial cell-derived autocrine/paracrine vasodilatory factors may be as important as effects on humoral factors. Indeed, previous studies have reported improvements in endothelial function with long-term VPI treatment.¹⁵

Our study has several limitations. We did not determine the humoral profile of hypertensive dogs before cardiac instrumentation. We did not administer ACE-I together with ADM. Though unlikely, it is possible that the combination would have resulted in more potent effects. The role of bradykinin and other endothelial factors stimulated by bradykinin was not explored. Because these factors function in an autocrine/paracrine role, a different experimental design will be required to investigate their relative role in the effects of VPI. The number of dogs in the long-term study was small and yet the major short-term findings, reduction of preload, augmentation of ADM and a lack of augmentation of natriuretic peptide levels were confirmed.

In experimental renal hypertension, short-term and long-term VPI therapy results in a rapid and persistent hypotensive effect that is mediated by short-term arterial vasodilation and dramatic, sustained reductions in preload. The humoral data suggest that the potent effects of VPI are related to a unique synergism of humoral modulation in hypertension or to the local augmentation of endothelial factors. Although our study does not fully establish the humoral mechanisms that mediate the unique hemodynamic responses to VPI, these data provide insight into the role of the natriuretic peptides, ADM, and the renin-angiotensin system and underscore the complexity of this novel and potent class of antihypertensive agents.

Perspectives

The current study provides insight into the complexity of actions of VPI therapy in hypertension. An adequate understanding of the specific hemodynamic and neurohumoral mechanisms of action of this new class of drugs suggests unique features that could potentially result in preferential cardioprotective effects. Further studies will be required to confirm these exciting hypotheses and to assess their relevance at the clinical level.

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