Vasopeptidase Inhibition Has Potent Effects on Blood Pressure and Resistance Arteries in Stroke-Prone Spontaneously Hypertensive Rats

Hope D. Intengan, Ernesto L. Schiffrin

Abstract—The antihypertensive agent omapatrilat represents a novel approach to antihypertensive therapy, namely vasopeptidase inhibition. Omapatrilat (BMS-186716) concomitantly inhibits neutral endopeptidase and angiotensin-converting enzyme, leading to protection from degradation of natriuretic and other hypotensive peptides in addition to interruption of the renin-angiotensin system. Although the potency of omapatrilat on reduction of blood pressure has been reported, its effects on resistance artery structure and function were unknown. We tested omapatrilat in stroke-prone spontaneously hypertensive rats (SHRSP), a malignant model of hypertension, with the hypothesis that it would improve the structure and endothelial function of mesenteric resistance arteries. Ten-week-old SHRSP were treated orally for 10 weeks with omapatrilat (40 mg/kg per day). Mesenteric arteries (lumen, 300 μm) were studied on a pressurized myograph. After 10 weeks, untreated SHRSP had a systolic blood pressure of 230 ± 2 mm Hg that was significantly reduced (P<0.05) by omapatrilat (145 ± 3 mm Hg). Omapatrilat treatment improved endothelium-dependent relaxation of resistance arteries as elicited by acetylcholine (10⁻² mol/L) but had no significant effect on endothelium-independent relaxation produced by a nitric oxide donor (sodium nitroprusside). This suggested that there existed endothelial dysfunction in SHRSP that was corrected by vasopeptidase inhibition, probably in part caused by the potent blood pressure–lowering effect of omapatrilat. Media width and media/lumen ratio were significantly decreased (P<0.05) by omapatrilat, and a trend (P=0.07) to increase lumen diameter was observed. Vascular stiffness (slope of the elastic modulus versus stress curve) was unaltered by omapatrilat. In conclusion, omapatrilat, acting as a potent antihypertensive agent, may improve structure and endothelial function of resistance arteries in SHRSP, a severe form of genetic hypertension. (Hypertension. 2000;35:1221-1225.)

Key Words: hypertension, malignant ■ vasculature ■ angiotensin-converting enzyme ■ natriuretic peptides ■ rats, stroke-prone SHR ■ remodeling

A novel basis for treatment of hypertension has recently been introduced in which a single molecule may modulate multiple physiological systems. One example of this treatment rationale is the vasopeptidase inhibitor omapatrilat (BMS-186716), a mercaptaoacetyl-based, fused heterocyclic dipeptide mimetic, which is a potent inhibitor of neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE). These enzymes are involved in the metabolism of several peptides that modulate blood pressure and sodium homeostasis, including angiotensin II, atrial natriuretic peptide (ANP), and bradykinin.

The stroke-prone hypertensive rat (SHRSP) model is a genetic model of malignant hypertension with an angiotensin-dependent component. ACE inhibitors significantly reduced systolic blood pressure in this model whether administered peripherally or centrally. Indeed, the conversion of angiotensin I to angiotensin II was markedly augmented in both spontaneously hypertensive rats (SHR) and SHRSP compared with Wistar-Kyoto rats. ACE also inactivates bradykinin and therefore ACE inhibition, by contributing to the accumulation of bradykinin, may facilitate the improved cardiac function and the vasodilatory effects of this hormone that otherwise appear normal in SHRSP.

The effects of NEP inhibition in SHRSP are less well studied, and the role that natriuretic peptides may play in the pathophysiology of SHRSP is unclear. Secretion of ANP from the heart is increased in SHR and SHRSP, as is gene expression of guanylate cyclase-A receptor in the kidney. However, in deoxycorticosterone acetate (DOCA)-salt but not SHR, NEP inhibition increased diuresis and natriuresis as well as urinary cGMP, ANP, and bradykinin. Nonetheless, the blood pressure–lowering effect of ACE inhibition was potentiated in SHR but not DOCA-salt rats by concomitant blockade of both enzymes.

We tested the hypothesis that vasopeptidase inhibition would have potent blood pressure–reducing effects and
would significantly improve endothelial function and structure of resistance arteries in SHRSP. To assess endothelial function, we evaluated vasodilatory responses to acetylcholine, which are impaired in SHR.\textsuperscript{13}

**Methods**

**Animals**
The study was conducted according to recommendations from the Animal Care Committee of the Clinical Research Institute of Montreal and the Canadian Council of Animal Care. Male SHRSP were obtained from a colony originally acquired from the National Institutes of Health and maintained locally. They were housed at 22°C and 60% humidity under a 12-hour light/dark cycle. Starting at 10 weeks old, rats were fed powdered diets (Purina Chow) containing omapatrilat (40 mg/kg per day, a dose that exerts a blood pressure–lowering effect equivalent to that of 10 mg/kg per day enalapril). Omapatrilat was kindly provided by Dr James R. Powell from Bristol-Myers Squibb.

Systolic blood pressure was measured biweekly by the tail-cuff method after rats were warmed and under slight restraint and recorded on a model 7 polygraph fitted with a 7-P8 preamplifier and PCPB photoelectric pulse sensor (Grass Instruments Co). The average of 3 pressure readings was obtained.

**Preparation of Resistance Arteries**
Rats were killed at 20 weeks of age by decapitation, and the mesenteric vasculature was dissected. Superior mesenteric arteries were taken from the part of the mesenteric vascular bed that feeds the jejunum 8 to 10 cm distal to the pylorus and placed in cold physiological salt solution (PSS) of the following composition: NaCl 120 mmol/L, NaHCO\textsubscript{3} 25 mmol/L, KCl 4.7 mmol/L, KH\textsubscript{2}PO\textsubscript{4} 1.2 mmol/L, MgSO\textsubscript{4} 1.2 mmol/L, CaCl\textsubscript{2} 2.5 mmol/L, EDTA 0.026 mmol/L, and glucose 5.5 mmol/L. A third-order branch of the mesenteric arterial tree (~2 mm in length) was carefully dissected 1 mm from the intestine and cleaned of all adherent connective tissue under a dissecting microscope. The arterial segments were mounted in a pressurized myograph chamber as previously described\textsuperscript{14} and under a dissecting microscope. The arterial segments were mounted

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**Endothelial Function Studies**
Endothelium-dependent relaxation was assessed by measuring the dilatory response to cumulative doses of acetylcholine (10\textsuperscript{-4} to 10\textsuperscript{-5} mol/L) of resistance arteries precontracted with norepinephrine (10\textsuperscript{-5} mol/L). Endothelium-independent relaxation was assessed by measuring the dilatory response to cumulative doses of sodium nitroprusside (10\textsuperscript{-7} to 10\textsuperscript{-4} mol/L) of small arteries precontracted with norepinephrine (10\textsuperscript{-5} mol/L).

**Vascular Morphology Studies**
To eliminate myogenic tone, mesenteric resistance arteries were deactivated by extraluminal perfusion with Ca\textsuperscript{2+}-free PSS containing 10 mmol/L EGTA for 30 minutes. Lumen and media dimensions were measured while the intraluminal pressure was maintained at 45 mm Hg.

**Vascular Mechanics**
Arterial wall stiffness was assessed by increasing intraluminal pressure stepwise up to 140 mm Hg, as previously described,\textsuperscript{15} and measuring media thickness and lumen diameter at 5 points along the vessel wall. The initial diameter was measured at 3 mm Hg unless the vessel collapsed. In these cases, intraluminal pressure-lumen diameter data (from 10 to 140 mm Hg) were fit to a third-order polynomial equation, and lumen diameter was estimated.

**Figure 1.** Systolic blood pressure of SHRSP over 10-week treatment period, treated orally with omapatrilat (40 mg/kg per day, n=6) or not treated. Error bars indicate SEM. *P<0.05.

**Calculation of Morphology and Mechanics**
For definitions of parameters, see Reference 16. Formulas used were:

\[
P_{z} = \frac{P_{o} - P_{z}}{\left(\frac{D_{o}}{D}\right)^{2} - 1},
\]

where \(P_{z}\) and \(P_{o}\) are the given intraluminal pressure and D\textsubscript{o}, respectively; Circumferential Stress \(\sigma = (P - D_{o})(D_{o})^{2} / (2 M)\), where P was the intraluminal pressure (dyne/cm\textsuperscript{2}) and D and M were lumen diameter and media thickness, respectively. Elastic Modulus was determined by fitting stress-strain data to \(\sigma = \sigma_{e} \beta \epsilon^{\beta}\), where \(\sigma_{e}\) was stress at D\textsubscript{o}, and \(\beta\) is a constant related to the rate of increase of the stress-strain curve. Tangential elastic modulus (ET) was calculated at several values of stress from the derivative of the exponential curve: ET = d\sigma/d\epsilon = \beta \sigma_{e} \epsilon^{\beta}.

**Data Analysis**
Data are presented as mean±SEM. Nonrepeated measurements were compared by means of the unpaired Student’s t test. Repeated data including mechanics were compared by means of ANOVA for repeated measures. Interaction means were analyzed for “simple main effects” with the use of a Student’s t test for unpaired data. A level of P<0.05 was considered significant.

**Results**

**Body Weight and Blood Pressure**
Untreated SHRSP weighed significantly less (305±4 g) compared with SHRSP treated with omapatrilat (319±2 g) (P<0.01), a difference that developed progressively since the beginning of treatment, as we have found in previous studies with effective antihypertensive treatment of hypertensive rats. After 2 weeks of treatment, systolic blood pressure was significantly reduced by omapatrilat, which was maintained for the duration of the study (Figure 1).

**Relaxatory Function of Mesenteric Resistance Arteries**
The vasodilatory response to acetylcholine, which is a measure of endothelium-dependent relaxation, was assessed. As depicted in the left panel of Figure 2, acetylcholine (10\textsuperscript{-5} mol/L) produced a 64.0±14.0% relaxatory response in mesenteric resistance arteries from untreated SHRSP that was improved by omapatrilat treatment (101.5±2.5%; P<0.05).
Omapatrilat had no significant effect on endothelium-independent relaxation (Figure 2) produced by a nitric oxide donor (sodium nitroprusside), which, at the highest dose of $10^{-4}$ mol/L, was 73.5±6.6% and 76.8±14.1% for untreated and omapatrilat-treated SHRSP, respectively.

**Morphology and Mechanics of Mesenteric Resistance Arteries**

Omapatrilat resulted in a tendency to increase lumen diameter ($P=0.07$) and decrease media width (from 17.5±0.9% to 15.0±0.6%, $P<0.05$) and media/lumen ratio (from 9.7±0.1% to 6.6±0.6%, $P<0.05$) in mesenteric resistance arteries from SHRSP (Figure 3). Stiffness of resistance artery wall components (slope of the elastic modulus versus stress curve) was unaltered by omapatrilat (Figure 4).

**Discussion**

Simultaneous inhibition of NEP and ACE with omapatrilat had potent effects on systolic blood pressure in SHRSP, reducing it by ≈85 mm Hg. The novel finding, however, is that omapatrilat also significantly improved endothelial relaxatory function and decreased media width and media/lumen ratio of mesenteric resistance arteries from SHRSP.

In this study, omapatrilat reduced blood pressure significantly in SHRSP. Indeed, vasopeptidase inhibition with omapatrilat appears to have potent antihypertensive effects in various rat models of hypertension as well as in normo- and hypertensive humans. In general, ACE inhibition is effective antihypertensive therapy in high-renin and normal-renin models such as renovascular rats and SHR, respectively, but less so in low-renin models such as DOCA-salt rats. On the contrary, NEP inhibitors are only effective in the latter, in which NEP inhibition increased diuresis and natriuresis as well as urinary cGMP, ANP, and bradykinin but failed to do so in SHR. Nevertheless, despite its lack of efficacy alone in SHR, NEP inhibition synergistically potentiated the blood pressure-lowering effect of ACE inhibition in SHR but not in DOCA-salt rats. The mechanism of action of omapatrilat is dichotomous, in which one arm is interruption of the renin-angiotensin system and the other is accumulation of natriuretic factors and vasodilatory peptides, such as bradykinin. Since both NEP and ACE inactivate bradykinin, perhaps combined NEP/ACE inhibition results in more complete protection of bradykinin, thereby explaining the potent effect of omapatrilat on blood pressure.

Vasopeptidase inhibition improved acetylcholine-induced, endothelium-dependent relaxation in mesenteric resistance arteries from SHRSP.
inhibitors of the renin-angiotensin system. One possible reason for the extended effect of omapatrilat on media/lumen ratio may be due to its distinctively potent effect on blood pressure as discussed above and may be at least in part attributed to NEP inhibition. Admittedly, NEP inhibition may result in decreased degradation of endothelin, as evidenced by an increase in circulating endothelin levels, and endothelin has been implicated in the hypertrophic remodeling typically detected in SHRSP. In an experimental model of pulmonary hypertension, NEP inhibition with the use of candesartan nonetheless reduced pulmonary vascular remodeling.

ANP has also been shown in vivo to inhibit hypertrophy of rat aortic smooth muscle cells. Marumo et al. reported that cytokine-induced nitric oxide production was significantly increased by ANP, brain natriuretic peptide, and CNP in rat aortic smooth muscle cells, accompanied by increased inducible nitric oxide synthase messenger levels. The resulting increase in nitric oxide may prevent vascular remodeling by the nature of its antigrowth effects. Moreover, CNP, which is produced also by vascular endothelial cells, exerts growth-inhibitory actions and is able to antagonize the growth-promoting effect of angiotensin II. The present findings combined with these purported antigrowth effects of natriuretic peptides suggest that increases in the hypertrophic peptide endothelin by NEP inhibition may be overcome by the antigrowth effects of natriuretic peptides. It should be noted that in contrast to small artery structure, which clearly appears to be corrected in rats in correlation with the degree of blood pressure lowering (Figure 5), when antihypertensive agents correct endothelial dysfunction in hypertensive rats, this occurs to a maximum degree whenever structure is improved independent of the degree of blood pressure normalization. This suggests that in rats, correction of endothelial dysfunction is found more easily and completely than for arterial structure, which is somewhat different from human studies.

In conclusion, this report provides further evidence that vasopeptidase inhibition in SHRSP results in potent antihypertensive effects and moreover produces significant vascular protection in the form of improved endothelial function and reduction of media/lumen ratio of resistance arteries. These actions of omapatrilat may confer protection against the extensive end-organ damage characteristic of severe or malignant hypertension.

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