Effect of ACE/NEP Inhibition on Cardiac and Vascular Collagen in Stroke-Prone Spontaneously Hypertensive Rats

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Left ventricular remodeling in hypertension is associated with cardiac interstitial and perivascular collagen deposition. The dual angiotensin I converting enzyme/neutral endopeptidase inhibitor omapatrilat (also called vasopeptidase inhibitor) improves left ventricular remodeling in experimental heart failure. We hypothesized that omapatrilat would induce regression of cardiac and vascular fibrosis in hypertension. We, therefore, investigated the effect of omapatrilat on collagen deposition in heart and aorta of stroke-prone spontaneously hypertensive rats (SHRSP). Twenty-week-old normotensive Wistar-Kyoto (WKY) rats, untreated SHRSP, and SHRSP treated with omapatrilat (40 mg/kg per day, orally) for 10 weeks were investigated. Collagen in the heart and the descending thoracic aorta was stained with Sirius red. After 10 weeks, systolic blood pressure (BP) was significantly (P < .01) reduced in omapatrilat-treated versus untreated SHRSP. Interstitial collagen density was significantly decreased in the subendocardial myocardium (to 2.71 ± 0.24% v 4.12 ± 0.30%, respectively, P < .05) and in the mid-myocardium of omapatrilat-treated versus untreated SHRSP (to 3.01 ± 0.25 v 4.19 ± 0.17% respectively, P < .05). Perivascular collagen was significantly (P < .05) decreased in the subepicardial, mid-myocardial and, sub-endocardial regions of the myocardium of omapatrilat-treated versus untreated SHRSP. Aortic collagen content decreased in omapatrilat-treated versus untreated SHRSP (to 36.1 ± 2.8 v 58.8 ± 6.1 × 10²µm²/mm section, respectively, P < .05). In conclusion, in addition to being a potent antihypertensive agent, omapatrilat significantly improves cardiac and vascular fibrosis in SHRSP. Am J Hypertens 2001;14:1067–1072 © 2001 American Journal of Hypertension, Ltd.

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BP\textsuperscript{16,17} and regress cardiomyocyte hypertrophy and perivascular fibrosis\textsuperscript{18} in this model. However, the effects of NEP inhibition in SHRSP are less well studied. Cardiac ANP is increased in SHR and SHRSP.\textsuperscript{19,20} Omapatrilat demonstrates antihypertensive effects in low, normal, and high renin models of hypertension.\textsuperscript{21,22} In experimental hypertension and in animal models of heart failure, omapatrilat improves cardiac performance and ventricular remodeling.\textsuperscript{23,24} Omapatrilat has potent BP-reducing effects in SHRSP and significantly improves endothelial function and structure of resistance arteries.\textsuperscript{25}

The aim of this study was to evaluate the effect of the dual inhibitor of NEP and ACE on interstitial and perivascular fibrosis in the heart, and on aortic collagen deposition in SHRSP. The hypothesis was that dual inhibition of NEP and ACE by omapatrilat would significantly improve ventricular and aortic fibrosis.

**Methods**

The study was conducted according to recommendations of 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 (NIH), and maintained locally. Rats were housed at 22°C and 60% humidity under a 12-h light/dark cycle. Starting at 10 weeks of age, SHRSP were fed powdered diet (Purina Chow) containing omapatrilat (40 mg/kg/day). SHRSP were killed by decapitation at 20 weeks of age. Normotensive Wistar-Kyoto (WKY 20-week-old) rats served as control group. Omapatrilat was kindly provided by Dr. James R. Powell (Bristol-Myers Squibb, Princeton, NJ).

Hearts and the descending thoracic aorta were dissected out and fixed in Bouin’s solution. After dehydration, the left ventricle and the aorta were embedded in paraffin. Serial sections (5 \( \mu \)m) were obtained, dewaxed with ethanol, and stained with Sirius red F3BA (Aldrich Chemical Company, Inc., Milwaukee, WI). Collagen deposition in the heart was evaluated throughout the inner third (subendocardial myocardium), the middle third (mid-myocardium), and the outer third (subepicardial myocardium) of the wall of the left ventricle. From each of three nonconsecutive serial sections (which allowed convergence of results), 30 fields in each region of the heart (magnification, \( \times20 \)) were recorded. The severity of cardiac fibrosis was evaluated after Sirius red staining with the use of an image analysis system (Northern Eclipse 5.0, EMPIX Imaging Inc., Mississauga, ON, Canada). Collagen density was defined as the ratio of the area stained by Sirius red to the area of the studied field (expressed as percentage). Perivascular collagen was excluded from this analysis and was measured separately. The perivascular collagen area was normalized to vessel luminal area of intramural coronary arteries.\textsuperscript{3} Only intramyocardial vessels that appeared circular on cross-section were analyzed. Total collagen content per millimeter of aortic section was calculated as the product of media surface area per millimeter of longitudinal section and collagen density: medial thickness in micrometer \( \times1000 \times \)collagen.\textsuperscript{26} The investigator responsible for the morphometric analysis was blinded to the experimental group being analyzed.

Results are expressed as mean ± SEM. Statistical significance was assessed by one-way ANOVA followed by a Student-Newman-Keuls test. Differences were considered significant at \( P < .05 \).

**Results**

**BP**

After 2 weeks of treatment with omapatrilat, systolic BP was significantly reduced in SHRSP, and remained lower for the duration of the study (Fig. 1).

**Interstitial Collagen Deposition in Left Ventricle**

The interstitial collagen density was significantly increased in the subendocardial (4.12 ± 0.30%) and in the mid-myocardial regions (4.19 ± 0.17%) of the left ventricle of SHRSP (Figs. 2 and 3A) compared to normotensive controls (2.27 ± 0.26% and 2.51 ± 0.35%, respectively, \( P < .05 \)). In contrast, in the subepicardial myocardium, interstitial collagen density was not significantly different compared to control. Treatment with omapatrilat decreased collagen deposition in the subendocardial and mid-myocardial regions of the left ventricle of SHRSP (2.71 ± 0.24% and 3.01 ± 0.25%, respectively, \( P < .05 \) v untreated SHRSP).
Perivascular Collagen Area in Left Ventricle
Compared to controls, the perivascular collagen area ratio (the relation between perivascular collagen and luminal areas of intramyocardial coronary arteries) was significantly increased in the subendocardial (5.13 ± 0.31 vs 1.83 ± 0.28, P < .01), mid-myocardial (4.65 ± 0.60 vs 2.03 ± 0.19, P < .01), and subepicardial regions (5.45 ± 0.91 vs 2.63 ± 0.18, P < .01) of the left ventricle of SHRSP (Figs. 3B and 4, left panels). A significant accumulation of collagen was also seen in the adventitia of intramyocardial coronary arteries from SHRSP. Treatment with omapatrilat significantly decreased perivascular collagen deposition in the left ventricle in the subendocardial (2.79 ± 0.41, P < .01), the mid-myocardial (2.34 ± 0.26, P < .05), and subepicardial regions of the myocardium (3.16 ± 0.43, P < .05).

Fibrosis of the Media of Thoracic Aorta
The aortic media thickness was significantly increased in SHRSP compared to controls (130.75 ± 4.37 μm vs 99.58 ± 5.41 μm, P < .05). Treatment with omapatrilat decreased aortic media thickness (95.96 ± 3.53 μm, P < .05 vs untreated SHRSP). In parallel, aortic collagen content was increased in SHRSP compared to controls (53.83 ± 6.11 × 10³ μm²/mm section vs 23.00 ± 2.79 × 10³ μm²/mm section, P < .05) and was reduced under omapatrilat treatment (36.05 ± 2.83 × 10³ μm²/mm section, P < .05 vs untreated SHRSP, Figs. 3c and 4, right panels).

Discussion
The present study investigated the effect of dual inhibition by omapatrilat of ACE and NEP on collagen deposition in the heart and aorta of SHRSP. In this genetic model of malignant hypertension both left ventricular interstitial collagen density and perivascular collagen content were dramatically increased in the left ventricle. Ten-week treatment with omapatrilat significantly reduced systolic BP by approximately 85 mm Hg, and significantly decreased cardiac interstitial and perivascular fibrosis and aortic collagen deposition.

Dual inhibitors of NEP and ACE have potent antihypertensive effects in low, normal, and high renin forms of experimental hypertension. However, selective inhibitors of ACE are particularly effective antihypertensive agents in the high-renin renovascular hypertensive rats and normal-renin SHR, but are ineffective in low-renin, deoxycorticosterone acetate (DOCA)-salt hypertensive rats. In contrast, NEP inhibitors are only effective in low-renin models of hypertension. Nevertheless, despite its lack of efficacy alone in SHRSP, NEP inhibition synergistically
potentiated the lowering BP effect of ACE inhibition in SHR but not in DOCA-salt rats.28

Dual NEP/ACE inhibition significantly reduced cardiovascular fibrosis in SHRSP, extending results of Farina et al.29 in the spontaneously hypertensive rat. Moreover, omapatrilat reduced perivascular fibrosis in the heart as well as in aorta. The potent BP-lowering effect of omapatrilat may be an important contributing mechanism to its antifibrotic effect. In TGR m(Ren2)27 rats, Bishop et al.7 demonstrated that elevated BP and not the renin-angiotensin system plays a causal role in cardiac fibrosis in this model. The NEP inhibition may also contribute, at least in part, to the antifibrotic effect of omapatrilat. Neutral endopeptidase inactivates the degradation of natriuretic peptides, such as ANP and BNP produced by cardiomyocytes. Atrial natriuretic peptide inhibits hypertrophy of rat aortic smooth muscle cells in vitro.30 In a study of cardiac fibrosis in mice lacking BNP, Tamura et al.11 showed that BNP is a cardiomyocyte-derived antifibrotic factor in vivo and plays a role as a local regulator of ventricular remodeling. By using cultured cardiac fibroblasts from neonatal rats, Fujisaki et al.13 demonstrated that natriuretic peptides interact with endogenous ET-1 in the regulation of cardiac fibrosis. Indeed, Cao et al.12 showed that natriuretic peptides played an important paracrine role in regulating fibroblast growth during cardiac hypertrophy. Moreover, c-type natriuretic peptide, which is produced by vascular endothelial cells, exerts growth-inhibitory actions and is able to antagonize the growth-promoting effect of angiotensin II.31 Neutral endopeptidase also plays a role in the metabolism of BK.32 Bradykinin reduces cardiac fibroblast procollagen synthesis.33 Because both NEP and ACE participate in the inactivation of bradykinin, their combined blockade may lead to a greater enhancement of bradykinin’s cardioprotective effect.32,34

Blockade of formation of angiotensin II by ACE inhibition may also participate in the antifibrotic effect of omapatrilat. Angiotensin converting enzyme inhibitors prevent cardiac remodeling18 as well as the increased deposition of collagen in the aorta in hypertension.35 In SHRSP, ACE inhibitors regressed cardiomyocyte hypertrophy, perivascular fibrosis, and medial thickening of intramyocardial coronary arteries.18 Schwartzkopff et al.36 recently showed that the ACE inhibitor perindopril induced regression of periarteriolar fibrosis and improved coronary reserve of patients with hypertensive heart disease.

Admittedly, NEP inhibition may result in decreased degradation of ET. Endothelin has been implicated in the hypertrophic remodeling typically detected in SHRSP.37,38 The involvement of ET-1 in cardiac fibrosis, demonstrated in DOCA-salt hypertensive rats,10 suggests that if NEP inhibition does result in increase of ET-1, this effect is not important, as the increase of ET-1 is counteracted by enhanced concentrations of ANP, BNP, BK, adrenomedullin, all of which have been shown to antagonize the effects of ET-1.39 Neutral endopeptidase inhibitors may owe their potent antihypertensive effects to enhancement of concentrations of endogenous natriuretic peptides,40 which could offset in part the activation of the renin-angiotensin and sympathetic nervous systems. Thus, association of ACE with NEP may allow expression of the antiproliferative as well as BP-lowering actions of various vasodilators. Moreover, Fujisaki et al.13 showed that natriuretic peptides inhibit angiotensin II-induced proliferation of rat cardiac fibroblasts by blocking ET-1 gene expression. The effects on cardiac and vascular fibrosis may be complex, with antifibrotic effects of natriuretic peptides associated to the inhibitory action.32,34

In conclusion, dual NEP/ACE inhibition with omapatrilat induces regression of cardiac and vascular fibrosis in
SHRSP. Dual inhibition of ACE and NEP with a vasopeptidase inhibitor may confer protection against cardiovascular remodeling in severe or malignant hypertension, and may become a useful and powerful addition to our current therapeutic approaches in the management of cardiovascular disease.

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References


FIG. 4. Perivascular collagen deposition around an intramyocardial coronary artery of the left ventricle (left) and in descending thoracic aorta (right) stained with Sirius red. Original magnification, ×20. Abbreviations as in Figs. 1–3.


