Effect of vasopeptidase inhibitor omapatrilat on cardiomyocyte apoptosis and ventricular remodeling in rat myocardial infarction

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Abstract

We have shown earlier that cardiomyocyte apoptosis continues at a high level late after myocardial infarction and contributes to adverse cardiac remodeling. Here we studied whether this process can be inhibited by the vasopeptidase inhibitor omapatrilat, a drug which causes simultaneous inhibition of both angiotensin converting enzyme and neutral endopeptidase. Our hypothesis was that omapatrilat-treated rats would have less cardiomyocyte apoptosis, and less adverse cardiac remodeling compared to rats treated with selective inhibitors of angiotensin converting enzyme, neutral endopeptidase or placebo. Myocardial infarction was produced by ligation of the left anterior descending coronary artery. Rats were randomized to receive omapatrilat, captopril, neutral endopeptidase inhibitor SQ-28603 or vehicle. Rats treated with omapatrilat and captopril had reduced cardiac BNP mRNA levels and less myocardial fibrosis by comparison with the vehicle-treated rats. However, omapatrilat was more effective than captopril in attenuating hypertrophy as measured by relative cardiac weight (3.0 ± 0.2 vs. 3.8 ± 0.2 mg/g, P < 0.01) or by echocardiographically determined left ventricular mass (0.61 ± 0.05 vs. 0.83 ± 0.06 g, P < 0.01). Myocardial apoptosis was elevated both in the infarction border zone (0.129 ± 0.017%) and in the remote area (0.035 ± 0.005%) still 4 weeks after myocardial infarction. Angiotensin converting enzyme inhibition proved to be important in the prevention of apoptosis since both omapatrilat and captopril reduced the number of apoptotic myocytes whereas selective neutral endopeptidase inhibitor SQ-28603 had no effect. In conclusion, myocardial apoptosis, remaining increased 4 weeks after myocardial infarction, was reduced by angiotensin converting enzyme inhibition. Vasopeptidase inhibition was more effective than selective angiotensin converting enzyme inhibition in preventing adverse cardiac remodeling after myocardial infarction.

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Keywords: ACE inhibitors; Apoptosis; Fibrosis; Hypertrophy; Infarction; Remodeling

1. Introduction

The neurohormonal activation after myocardial infarction (MI) plays an important role in the development and progression of adverse cardiac remodeling. The modulation of this neurohormonal response is an important target in preventing development of heart failure [1–3]. Treatment with angiotensin converting enzyme (ACE) inhibitors and angiotensin II (AT II) type 1 receptor (AT1) blockers has been shown to inhibit cardiac hypertrophy, preserve cardiac function, and attenuate changes in myocardial gene expression in experimental MI models [4]. The beneficial effect of renin–angiotensin system blockade on survival...
after MI has also been demonstrated in multiple clinical trials [5,6].

Myocardial remodeling after MI includes compensatory hypertrophy of the cardiac myocytes [7–9], but also an increase in interstitial collagen deposition [10]. Transition from compensatory hypertrophy to progressive dilatation and heart failure is mediated at least partly by the continuous loss of myocytes due to apoptosis [11–14]. In experimental MI models myocardial apoptosis has been shown to continue at a high level not only in the areas adjacent to the infarction zone, but also in the more remote areas of the left ventricle (LV) [15–17]. This can be due to pressure overload [18] and neurohormonal factors, both of which have been demonstrated to induce apoptosis in the heart. AT II is a direct trigger of apoptosis of ventricular myocytes in vitro [19]. However, it is unclear whether post-MI apoptosis can be prevented by ACE-inhibitors or even more effectively by inhibiting neutral endopeptidase (NEP).

The objective of this study was to investigate the effects of combined ACE and NEP inhibition on adverse myocardial remodeling and apoptosis after MI. We used omapatrilat, a single molecule that simultaneously inhibits both NEP and ACE [20]. NEP inhibition protects atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and related peptides from enzymatic inactivation [21] and thus preserves the vasodilatory, natriuretic, diuretic [20], and antihypertrophic effects of these peptides [22]. Concurrent ACE inhibition blocks AT II formation and increases bradykinin levels [23–25]. The co-inhibition of NEP and ACE has been shown to produce greater haemodynamic and renal effects than inhibition of either NEP or ACE alone in several models of heart failure and hypertension [26,27]. Our hypothesis was that omapatrilat would have greater anti-apoptotic and anti-remodeling effects compared with the selective inhibitors of ACE or NEP in the rat MI model.

2. Methods

2.1. Animals

Male Wistar rats were obtained from the Viikki Biocenter, Helsinki, Finland. Animals were housed at 23–25 °C in a 12-h light/dark cycle with access to standard powdered rat chow and normal water. The Helsinki University Central Hospital Animals Ethics Committee and the Provincial State Office of Southern Finland approved the experimental procedures.

2.2. Drugs

Omapatrilat, a dual NEP and ACE inhibitor, SQ-28603, a selective NEP inhibitor and captopril, a selective ACE inhibitor, were provided by Bristol-Myers Squibb Pharma-ceuticals (Princeton, NY, USA). The drugs under study were mixed to powdered rat chow. The doses were selected according to our earlier dose–response work [28] and targeted at omapatrilat 40 mg/kg per day, SQ-28603 100 mg/kg per day, and captopril 100 mg/kg per day. The actual consumed doses were omapatrilat 39 mg/kg per day, SQ-28603 88 mg/kg per day and captopril 109 mg/kg per day.

2.3. Study outline

The animals were acclimated to powdered rat chow without medication for 5 days. Daily food and water consumption were determined throughout the study. The body weights were recorded before starting medication, before operation and 1 and 4 weeks after MI operation. The animals were randomised to the treatment groups 24 h before the MI operation to cover also the acute and subacute phases of infarction. The medication had no effect on the size of the infarction, since there was no difference in the infarcted area between the groups as measured by planimetry at 1 or 4 weeks after the operation. MI was produced by ligation of the left anterior descending coronary artery as described earlier [15,29]. The control rats underwent the same procedure, except for the ligation of the coronary artery (sham operation). Systolic blood pressure (SBP) was measured before starting the medication, 1 and 4 weeks after operation by tail cuff plethysmography in conscious lightly restrained animals. Echocardiography was carried out in all animals 4 weeks after operation. The animals were sacrificed 1 or 4 weeks after operation. Hearts that showed no histological signs of infarction were not included in the study. There were seven rats in each omapatrilat and captopril group, 6–8 rats in SQ-28603 groups and 10 rats in vehicle control groups.

2.4. Samples

The rats were sacrificed by phenobarbital 55 mg/kg (Mebunat®, Orion Pharma, Espoo, Finland) intraperitoneally and blood samples were collected into pre-chilled lithium heparin tubes and into tubes containing Na2EDTA and aprotinin (500 kallikrein inhibitor units per ml). Heart and kidney weights were determined. Heart and kidneys were snap-frozen in isopentane at −40 °C and stored at −80 °C for subsequent in vitro autoradiography analysis. Before freezing the heart was excited into 2-mm transverse slices distal to the level of coronary ligation. One of the myocardial slices was fixed in 4% neutral buffered formalin for 24 h, embedded in paraffin, and cut into 4-μm-thick sections for histology, planimetry, and assessment of apoptosis. Plasma renin activity (PRA), N-terminal ANP and BNP were measured by radioimmunoassay [28,30].
2.5. Autoradiographs

Quantitative in vitro autoradiography was carried out on renal sections (20 μm) with the radioligand [125I]RB104 for NEP and [125I]MK351A for ACE as described earlier [31,32]. The optical densities were quantified by an AIDA computer image analyzing system (AIDA 2D densitometry) coupled to the FUJIFILM BAS-5000 phosphoimager (Tamro, Finland). Specific binding was calculated as total binding minus non-specific binding.

2.6. Histology and planimetry of infarct size

The presence of signs of either acute MI or collagen scars was analyzed under microscope by examination of Van Gieson-stained transverse LV sections. Infarct size was determined planimetrically as the ratio of infarcted tissue or scar to the length of the entire LV endocardial circumference. Infarcts were classified as small (10–30%), moderate (31–49%) or large (>50%). There were no statistically significant differences in infarct sizes between the groups.

2.7. BNP mRNA

BNP mRNA expression was determined both from the border zone of MI and from the remote area of the LV. Total RNA was prepared with the QuickPrep® system (Amersham–Pharmacia Biotech, Uppsala, Sweden) and the cDNA first strand was synthesized using M-MuLV reverse transcriptase. The quantitative PCR reactions were carried out with an ABI 7700 Sequence Detection System using TaqMan® chemistry. The forward and reverse primers for rat BNP mRNA detection were TGGGCAGAAGATAGACCGGA and ACAACCTCAGCCCGTCACAG, corresponding to nucleotides 300–319 and 361–342 of rat BNP cDNA coding sequence, respectively. The 62 bp amplicon was detected using the bifunctional fluorogenic probe 5′-Fam™-CCAAGCGACTGACTGCGCCG-Tamra™-3′. The results were normalized to 18S RNA quantified from the same samples as described previously [33].

2.8. Myocardial fibrosis

The interstitial collagen volume fraction (CVF) was determined from three different slices of picrosirius red (Fluka)-stained LV sections under polarized light. Areas of connective tissue network and myocytes were quantified by a semiautomated computer-based analysis system as described by Brooks et al. [34].

2.9. Apoptosis

Apoptotic cardiomyocytes were detected with the terminal deoxynucleotide transferase-mediated ddUTP nick end labeling (TUNEL) assay. Analysis of apoptosis was carried out in one LV slice obtained from the sample showing maximal infarct size. The number of apoptotic cardiomyocytes was counted in the whole LV under a light microscope with an ocular grid. The apoptotic myocytes were identified by the presence of myofilaments surrounding the nucleus. The amounts of apoptotic cardiomyocytes were expressed as the proportion of TUNEL-positive cardiomyocyte nuclei from the total number of cardiomyocyte nuclei, obtained by multiplying the density of cardiomyocyte nuclei in a serial Dnase I-treated control section times the area of the section [11,15,35].

2.10. Echocardiography

Prior to echocardiography, the animals were sedated by medetomidine 0.25 mg/kg (Domitor®, Orion Pharma, Espoo, Finland) given subcutaneously and placed on a thermal plate. Two-dimensional echocardiographic measurements were carried out using a 7–10 MHz phased array sector probe (Toshiba Power Vision 8000, Toshiba Medical Systems, Nasu, Japan) from the right parasternal projection. An average of three measurements was used to assess LV end diastolic diameter (LVEDD), LV anterior wall thickness, and posterior wall thickness (PW). LV end diastolic volume (LVEDV) was measured by Simpson’s formula. LV mass was calculated from a standard cube formula and relative PW thickness (2*PW/LVEDV) as described [36]. The coefficient of variation was 3.3% for LVEDD, 8.7% for PW, and 7.9% for LVEDV, respectively.

2.11. Statistical methods

Results are presented as mean±S.E.M. unless otherwise stated. Data were analyzed using analysis of variance (ANOVA) including infarction size (MI%) as a covariate where appropriate. If statistical significance was obtained between groups, Fisher’s protected least significance test was used to determine where significant differences existed. Blood pressure measurements were analyzed using repeated measures ANOVA. A difference at P<0.05 was considered statistically significant.

3. Results

3.1. Body weight, systolic blood pressure, and hormonal parameters

3.1.1. Body weight

There was no statistical difference in the body weight between the groups either before the operation or after the 4-week treatment period (Table 1).
3.1.2. Blood pressure

Mean SBP before starting the medication was 129 mmHg. One week after MI, SBP was lower in the omapatrilat (91 ± 7 mmHg, \( P < 0.01 \)) and captopril (97 ± 5 mmHg, \( P < 0.05 \)) treated groups compared to the selective NEP inhibitor (113 ± 5 mmHg), but there was no significant difference by comparison with the vehicle MI group. Four weeks after the operation SBP was lowest in the omapatrilat group, but there was no significant difference compared to the captopril or vehicle-treated rats (Fig. 1).

3.1.3. Hormonal effects

Treatment with captopril but not with omapatrilat increased significantly PRA (Table 1). PRA level in captopril-treated rats was sevenfold compared to control MI rats (\( P < 0.001 \)) and over 2.5-fold compared to omapatrilat-treated rats (\( P < 0.001 \)). SQ-28603 had no effect on PRA. There were no significant differences in plasma N-terminal ANP or BNP levels between any of the treatment groups.

3.1.4. ACE and NEP autoradiography

ACE and NEP inhibitions from kidney samples were analyzed by quantitative autoradiography [26,32] to control the effectiveness of drug administration. The renal ACE autoradiography signal was 22% higher after 4 weeks in vehicle MI rats compared with sham-operated rats, but the difference was not statistically significant. There was no change in NEP autoradiography signal levels after MI in vehicle-treated rats. Omapatrilat showed inhibition of both ACE and NEP (remaining activities 30 ± 5%, \( P < 0.001 \) and 36 ± 6%, \( P < 0.001 \), respectively, Table 1). SQ-28602 inhibited NEP (36 ± 3%, \( P < 0.001 \)), but had no effect on ACE (94 ± 4%). Captopril had no inhibitory effect on renal NEP (98 ± 7%), but inhibited renal ACE (71 ± 4%, \( P < 0.01 \)).

3.2. Hypertrophy, BNP gene expression and fibrosis

3.2.1. Heart weight

A compensatory increase in the relative heart weight was detected 4 weeks after MI in non-treated rats (\( P < 0.01 \), Fig. 2A). At 1 week there were no differences in relative cardiac weights between the groups. However, 4 weeks after operation relative cardiac weights were significantly smaller in the omapatrilat (3.0 ± 0.2 mg/g, \( P < 0.01 \)) and SQ-28603 (3.3 ± 0.2 mg/g, \( P < 0.05 \)) treated groups compared to control MI rats (3.9 ± 0.3 mg/g) (Fig. 2B). Also as measured by echocardiography, LV mass was significantly lower in the omapatrilat-treated group compared to captopril and control MI groups (Table 2).

3.2.2. BNPmRNA

LV hypertrophy after MI involves the hypertrophic growth of cardiomyocytes and the accumulation of fibrillar collagen in the interstitium. As a marker of hypertrophic gene expression we used BNP mRNA levels. After MI, the expression of BNP mRNA increased both in the border zone of infarction and in the remote area compared to the

Fig. 1. Mean systolic blood pressures before operation, 1 and 4 weeks after MI in different treatment groups. There were no significant differences in SBPs between omapatrilat, captopril and vehicle-MI rats, though SBP was lowest in the omapatrilat-treated rats.
weeks the expression was lower in the omapatrilat and captopril-treated rats. The reduced BNP mRNA expression was detected both in border zone and remote areas (Fig. 3B). Instead, in the SQ-28603-treated group there were no statistical differences in BNP mRNA levels compared to the vehicle-treated MI group in either area.

3.2.3. Myocardial fibrosis

To estimate the interstitial component of the hypertrophy, we measured the levels of fibrosis from the LV tissue sections 4 weeks after the operation. CVF was determined from the non-infarcted area by quantitative morphometry. Myocardial fibrosis increased after MI. In the sham operated animals, CVF in the LV was 1.9±0.4%, whereas in the vehicle MI group, CVF was 4.8±0.9% (P<0.001) (Fig. 4). Both omapatrilat and captopril treatments decreased CVF compared to the vehicle-treated group (2.8±0.2%, P<0.01 and 2.8±0.7%, P<0.01, respectively). Selective NEP inhibition by SQ-28603 did not have any effect on fibrosis.

3.3. Apoptosis and left ventricular dilatation

3.3.1. Cardiomyocyte apoptosis

Apoptosis increased after MI both in the border zone and in the remote area. The apoptosis was 3.6-fold higher in the border zone compared with the remote area 4 weeks after MI. The percentage of apoptotic cardiomyocytes in the myocardium of sham operated rats was 0.01±0.002% (Fig. 5) and remained stable from 1 to 4 weeks. In the border zone areas of vehicle-treated infarctions, the percentage of apoptotic cells decreased from 0.18 to 0.13% (P=0.34) from 1 to 4 weeks. In the remote noninfarcted myocardium, apoptosis increased from 0.014% at 1 week to 0.035% at 4 weeks (P<0.05).

Both omapatrilat and captopril treatments decreased the number of apoptotic myocytes in LV tissue sections. After 1 week the level of apoptosis in the border zone area of MI was 0.14% with captopril-treated rats and 0.13% with omapatrilat-treated rats compared to 0.18% in vehicle-treated MI rats, but the difference was not statistically significant. After 4 weeks of treatment there was a significant decrease in apoptosis in the border zone of infarction in the omapatrilat-treated group compared to the vehicle group (0.06% and 0.13%, respectively, P<0.01, Fig. 5). A significant decrease in apoptosis was also detected in the captopril-treated animals both in the border and also in the remote zone (0.08%, P<0.05, and 0.019%, P<0.05, respectively). In contrast, the amount of apoptosis in the SQ-28603-treated animals did not differ from the control MI group 4 weeks after the operation.

LV dilatation after MI was evaluated by transthoracic echocardiography. The echocardiographically measured LV dimensions are summarized in Table 2. LV dilatation was observed 4 weeks after operation as the LVEDDs and LVEDVs were significantly larger in the vehicle MI group.

Fig. 2. Relative cardiac weight 1 and 4 weeks after MI, and effect of different treatments on cardiac weight 4 weeks after MI. (A) Cardiac weight increased gradually after MI and after 4 weeks the difference was statistically significant. The increase in cardiac weight is expressed as relative to 1 week sham operated rats. (B) Omapatrilat and SQ-28603-treated rats had lower relative cardiac weights compared to vehicle MI rats after 4 weeks of treatment. There was a statistically significant difference between omapatrilat and captopril-treated animals. Results are expressed as mean±S.E.M. **P<0.01. SQ-28603 stands for the selective NEP inhibitor.
Table 2
Echocardiographic parameters in MI operated rats 4 weeks after operation

<table>
<thead>
<tr>
<th></th>
<th>Sham MI</th>
<th>Vehicle+ MI</th>
<th>Omapatrilat+ MI</th>
<th>Captopril+ MI</th>
<th>SQ-28603+ MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass (g)</td>
<td>0.76±0.05</td>
<td>0.86±0.04</td>
<td>0.61±0.03**</td>
<td>0.83±0.06</td>
<td>0.73±0.05</td>
</tr>
<tr>
<td>PW thickening</td>
<td>0.32±0.02</td>
<td>0.27±0.01*</td>
<td>0.30±0.01</td>
<td></td>
<td>0.28±0.01*</td>
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<td>ratio</td>
<td></td>
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<tr>
<td>LVEDD (mm)</td>
<td>7.9±0.2</td>
<td>9.1±0.4*</td>
<td>8.6±0.4</td>
<td>9.1±0.2</td>
<td>9.6±0.3</td>
</tr>
<tr>
<td>LVEDV (µl)</td>
<td>566±34</td>
<td>692±48&quot;</td>
<td>604±72</td>
<td>690±48</td>
<td>752±76</td>
</tr>
</tbody>
</table>

Values are given as mean±S.E.M.

*P<0.05 Sham versus Vehicle+MI, **P<0.05 versus Vehicle+MI, ***P<0.01 versus Vehicle+MI.

when compared to sham operated rats, but there were no significant differences between the different MI treatment groups. However relative wall thickness decreased significantly both in omapatrilat and SQ-28603-treated rats compared with the vehicle MI rats (Table 2).

4. Discussion

Our results indicate that omapatrilat was more effective than captopril in preventing cardiac hypertrophy after experimental MI in rats. Omapatrilat as well as captopril reduced hypertrophic gene expression and had an antifibrotic effect, whereas SQ-28603 had no effect. Both omapatrilat and captopril treatments inhibited cardiomyocyte apoptosis.

4.1. Hemodynamic and hormonal effects of ACE/NEP inhibition

The objective of this study was to compare dual ACE/NEP inhibitor omapatrilat to selective ACE and NEP inhibitors, captopril and SQ-28603, respectively, in a rat MI model. Omapatrilat is the most clinically developed vasopeptidase inhibitor [37]. It produces a balanced inhibition of NEP and ACE in vivo [28], but has no effect on other related enzymes [38]. Both preclinical studies as well as clinical trials have shown that omapatrilat is effective in treating heart failure and it is suggested to have greater impact on morbidity and mortality than selective ACE inhibitors alone [39–42].

One of the adverse effects of the selective ACE inhibition is the compensatory increase in PRA. This increase may partially damp the beneficial hemodynamic and antihypertrophic effects of ACE inhibitors. In our study, captopril treatment induced an increase in PRA similar to what has been reported earlier [43]. However, despite the ACE inhibitory component of omapatrilat, PRA was not significantly increased in omapatrilat-treated animals compared with the animals treated with vehicle. The lack of the compensatory activation of the renin–angiotensin system may be explained by NEP inhibition, because enhanced action of natriuretic peptides has been reported to inhibit renin synthesis [44].

4.2. Effect of ACE/NEP inhibition on cardiac hypertrophy and BNP gene expression

BNP mRNA expression is a sensitive marker of LV hypertrophy [45,46,4]. In our study, BNP mRNA in the
border zone of infarction was already clearly increased 1 week after the MI operation in vehicle-treated rats. After 4 weeks, an increase in BNP mRNA was also detected in the remote noninfarcted area. Omapatrilat and also to a lesser extent captopril significantly decreased BNP mRNA expression both in the border zone and in the remote area of myocardium 4 weeks after MI. Selective NEP inhibition did not affect the BNP mRNA expression.

In accordance with the decreased BNP expression, omapatrilat treatment reduced relative cardiac weights as well as echocardiographically measured LV mass compared to vehicle-treated rats. It has been previously demonstrated that another vasopeptidase inhibitor S21402 reduced the mass of both ventricles of the heart after MI [43]. In that study captopril regressed LV mass, selective NEP inhibitor SCH42495 regressed right ventricular mass and the combined effect of these drugs was equal to the cardiac mass reduction caused by the vasopeptidase inhibitor S21402. In another study, candesartan and cilazapril both significantly prevented the increase in heart weight at 4 weeks after MI operation [47].

In our experimental setting, ACE inhibition alone did not cause significant changes in cardiac weight, which may depend on the relatively short follow-up time. Slightly
lower SBP levels with omapatrilat may contribute to the better antihypertrophic effect. Omapatrilat is an effective inhibitor of cardiac ACE as demonstrated by our earlier in vitro autoradiography findings [28] and this may also explain the more effective reduction in heart mass by omapatrilat compared to captopril treatment. Inhibition of NEP may also reduce hypertrophy by increasing autocrine and paracrine actions of natriuretic peptides, as a selective NEP inhibitor, SCH42495, reduced LV hypertrophy with minimal effects on blood pressure [48].

4.3. Effect of ACE/NEP inhibition on myocardial fibrosis

Both omapatrilat and captopril reduced myocardial fibrosis after MI. Collagen deposition during healing of the MI is central for preserving the strength of the infarcted area [49,50]. However, the accumulation of interstitial fibrosis to the noninfarcted area is associated with the development of hypertrophy and may have a negative effect on the diastolic function of the heart [51]. The renin–angiotensin system is an important regulator not only of cardiomyocyte hypertrophy but also of interstitial matrix. AT II stimulates cardiac fibroblast collagen synthesis via the AT1 receptor in vitro [52], and both ACE inhibition and AT1 blockade have been shown to inhibit the interstitial accumulation of collagen after MI and in hypertensive heart disease [51,53].

NEP inhibition reduces degradation of natriuretic peptides, which are known to have an antifibrotic effect by the
stimulation of their A- and B-type receptors [54]. ACE and NEP also act as potent kinin-degrading enzymes in plasma and tissues [55,56] and accordingly, plasma and tissue bradykinin levels are increased during ACE/NEP inhibition [57]. Kinins have been shown to contribute to the reduction of myocardial collagen accumulation by ACE inhibition after MI [58]. Farina et al. [59] have shown that dual NEP/ACE inhibitor S21402 inhibited cardiac fibrosis in spontaneously hypertensive rats. Both captorpril and selective NEP inhibitor treatments had antifibrotic effects. In the present work, the antifibrotic effect was similar with both omapatrilat and captorpril. However, we found no inhibition of the fibrosis with the selective NEP inhibitor SQ-28603 alone.

4.4. Effect of ACE/NEP inhibition on myocardial apoptosis

Experimental studies have shown that apoptosis is induced during hypoxia [60] and ischemia–reperfusion in myocardium [61]. Apoptosis is also activated in human acute MI [62]. We and others have earlier shown that cardiomyocyte apoptosis occurs continuously over an extended period of time both in the border zone of MI and in the remote, noninfarcted area [15,17].

Progressive deterioration of LV function and development of heart failure after MI is mediated in part by loss of cardiac myocytes due to apoptosis [14,15]. ACE inhibitor enalapril has been shown to decrease apoptosis in the border zones of infarct scars in a dog model of ischemic heart failure [63]. In our study, both omapatrilat and captorpril decreased apoptosis in the border zone of the infarct but also to a lesser extent in the remote noninfarcted area. In contrast, selective NEP inhibition did not affect the number of apoptotic cells. AT II has been earlier reported to directly induce apoptosis in isolated cardiac myocytes [19], but the precise mechanism is unknown. Another possible explanation for the decreased apoptosis by ACE-inhibitors is the reduced pressure load. This is supported by the findings of Teiger et al. [18] who demonstrated that pressure overload induces myocardial apoptosis in experimental rat aortic stenosis.

In conclusion, combined NEP/ACE inhibition with the vasopeptidase inhibitor omapatrilat, had several favorable effects in the post-MI model in the rat. Development of cardiac hypertrophy and myocardial interstitial fibrosis as well as acceleration of apoptotic cardiomyocyte loss after MI were effectively reduced in rats treated with omapatrilat. Combined NEP/ACE inhibition was more effective than selective ACE inhibition in reducing total cardiac mass in this model. NEP inhibition alone reduced cardiac hypertrophy only slightly. The introduction of vasopeptidase inhibitors provides a new therapeutic strategy that may confer improved cardiac protection compared to ACE inhibition in cardiac diseases.

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