Comparison of the Effects of an Angiotensin-Converting Enzyme Inhibitor and a Vasopeptidase Inhibitor After Myocardial Infarction in the Rat

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OBJECTIVES	The goal of this study was to compare the effects of the vasopeptidase inhibitor omapatrilat and the angiotensin-converting enzyme inhibitor (ACEI) captopril in the postmyocardial infarction (MI) rat model.
BACKGROUND	The cardioprotective effects of ACEIs after MI are thought to be partially due to an increase in bradykinin (BK). Vasopeptidase inhibitors inhibit both ACE and neutral endopeptidase (NEP), further reduce BK metabolism and increase natriuretic peptides, which may result in better cardioprotective effects than with ACEIs after MI.
METHODS	Myocardial infarction was induced in 514 Wistar male rats by ligation of the anterior coronary artery. Rats surviving 4 h after MI (n = 282) were assigned to omapatrilat (40 or 80 mg/kg/day), captopril (160 mg/kg/day) or no treatment. After 56 days, neurohumoral, hemodynamic, ventricular remodeling, morphometry, immunohistochemistry and cardiac cytokine expression were measured.
RESULTS	Omapatrilat and captopril resulted in similarly improved survival, cardiac hemodynamics and reduced cardiac fibrosis and hypertrophy after MI. The pattern of left ventricular (LV) remodeling differed, omapatrilat causing less attenuation of the rightward shift of the LV pressure-volume relation at lower filling pressures than captopril. Both interventions reduced messenger ribonucleic acid expression of the profibrotic cytokine transforming growth factor-β_1; neither effected the anti-inflammatory cytokine interleukin-10, and only captopril reduced the proinflammatory cytokine tumor necrosis factor-alpha (TNF-α). Expression of TNF-α was in cardiomyocytes. Both medications reduced circulating endothelin-1, angiotensin II and catecholamines, but only omapatrilat increased atrial natriuretic peptides.
CONCLUSIONS	This study indicates that both omapatrilat and captopril markedly improve post-MI survival, cardiac function and cardiac remodeling in the rat. It would appear that the addition of NEP inhibition to those of ACEIs does not result in significant further benefit after MI. (J Am Coll Cardiol 2002;39:1692–8) © 2002 by the American College of Cardiology Foundation

Angiotensin-converting enzyme inhibitors (ACEIs) have been shown to reduce cardiac remodeling and to prolong survival after myocardial infarction (MI) in experimental models and in patients (1). Although we initially thought that ACEIs exerted their beneficial effects exclusively by reducing the synthesis of angiotensin II (Ang II), there is now mounting evidence that they exert at least some of their beneficial effects by inhibiting the degradation of the endogenous vasodilator bradykinin (BK) as well (2).

A new class of medications that inhibit both the ACE and neutral endopeptidase (NEP) enzymes, called vasopeptidase inhibitors (VPI), further enhance nitric oxide and vasodilator prostaglandins and increase other endogenous vasodilators, such as natriuretic peptides and adrenomedullin, by blocking their metabolism (3). A recent study demonstrating beneficial effects of a 62-h infusion of atrial natriuretic peptide (ANP) in patients with an acute anterior MI would suggest that further enhancement of this endogenous vasodilator system is useful after MI (4). The VPI omapatrilat has a balanced effect on both the NEP and the ACE enzymes, with an inhibition constant in the nanomolar range for both enzymes (5). In the cardiomyopathic hamster, it has been shown to prolong survival more than the ACEI captopril (6), and, in the pacing overdrive dog model of heart failure, it has been shown to preserve myocardial contractility (7). In one clinical study of patients with heart failure, omapatrilat compared favorably with the ACEI lisinopril (5).

In this study, we compared the effects of the VPI omapatrilat on post-MI survival, ventricular function, remodeling and neurohumoral activation to those of the ACEI captopril. Because VPIs may also enhance systemic and local inflammatory responses by further increasing BK levels (8), we also measured cardiac messenger ribonucleic acid (mRNA) expression of several cytokines. Our hypothesis was that omapatrilat would exert greater beneficial effects on survival, ventricular function and remodeling than the ACEI captopril.

Abbreviations and Acronyms

ACEI	= angiotensin-converting enzyme inhibitor
Ang II	= angiotensin II
ANP	= atrial natriuretic peptide
AW	= atrial weight
BK	= bradykinin
BW	= body weight
ET-1	= endothelin-1
IgG	= immunoglobulin-G
IL	= interleukin
LV	= left ventricle/ventricular
LVEDP	= left ventricular end-diastolic pressure
LVSP	= left ventricular systolic pressure
LVW	= left ventricular weight
MI	= myocardial infarction
mRNA	= messenger ribonucleic acid
NEP	= neutral endopeptidase inhibitor
RT-PCR	= reverse transcriptase-polymerase chain reaction
RV	= right ventricle/ventricular
RVSP	= right ventricular systolic pressure
RVW	= right ventricular weight
TGF-β	= transforming growth factor-beta
TNF-α	= tumor necrosis factor-alpha
VPI	= vasopeptidase inhibitor
+dP/dt	= maximum rate of pressure rise

METHODS

MI. Myocardial infarction was induced in 514 male Wistar rats (Charles River, St-Constant, Quebec, Canada) weighing 200 g to 250 g through ligation of the left anterior descending coronary artery as previously described (9). All of the animal experiments followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Ethics Committee of the Montreal Heart Institute (Montreal, Quebec, Canada). The sham ligation group underwent a similar procedure except that the suture was not tightened around the coronary artery.

Drug randomization. The ACEI captopril and the VPI omapatrilat, which acts by combined inhibition of ACE (inhibitory concentration of 50% activity [IC_{50}] = 5 nM) and NEP (IC_{50} = 9 nM), were provided for research purposes by Bristol-Myers Squibb (Princeton, New Jersey). Rats were randomly divided into four groups (Fig. 1). An intraperitoneal injection of the different medications were administered 4 h after MI and the morning after MI. A first group received subcutaneous injections of saline followed by normal food (untreated group, n = 99). A second group received an intraperitoneal injection of omapatrilat 4 mg/kg and 40 mg/kg/day in food thereafter (n = 62) (7). A third group received an intraperitoneal injection of omapatrilat 8 mg/kg and 80 mg/kg/day in food thereafter (n = 56) (5). A fourth group received an intraperitoneal injection of captopril 16 mg/kg and 160 mg/kg/day in food thereafter (n = 65).

Cardiac hemodynamic measurements. After 56 days of therapy, the rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg) (Rogar/STB Montreal, Quebec, Canada) and xylazine (10 mg/kg) (Bayer Canada, Etobicoke, Ontario, Canada) mixture. An electrocardiogram was performed, and the left (LV) and right ventricular (RV) pressures were measured by a Millar Micro-Tip Catheter Transducer (Millar Instruments, Houston, Texas) as previously described (9).

Serum neurohumoral measurements. After the hemodynamic measurements were obtained, a catheter was introduced in the right jugular vein for blood sampling. Serum ANP, Ang II, endothelin-1 (ET-1) and catecholamines were measured by methods described previously (9).

Ventricular remodeling group. PASSIVE PRESSURE-VOLUME RELATION. After completing the cardiac hemodynamic measurements, 144 rats had their hearts stopped in
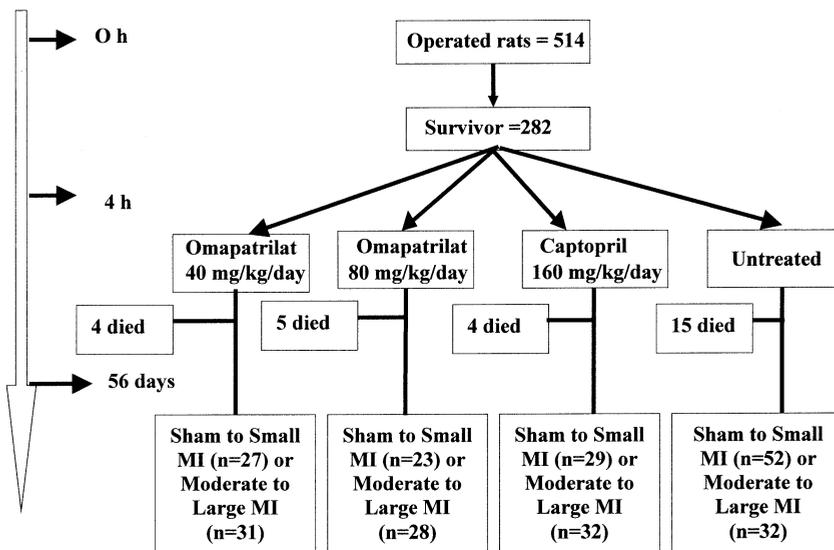

Figure 1. Flow diagram of various groups of rats according to the presence of myocardial infarction (MI) and treatment group. All rats were randomized 4 h after coronary artery ligation. The MI size was determined at the end of the study, and, thereafter, the rats were classified according to their MI size.

diastole, and three pressure-volume curves were obtained within 10 min; an average of these three curves was used as the final value, as previously described (9).

Morphologic characteristics of mid-LV cross-sections. Once the pressure-volume curve was completed, the LV was filled with saline solution to a pressure of 15 mm Hg, sealed and fixed in its distended form in 10% formalin phosphate buffer for 24 h. Right and left atria and both RV and LV weights were determined. Two cross-sections were obtained at 1-mm intervals midway between the base and the apex of the LV. Transverse sections of 4-μm thick were cut, and two cross-sections of each LV were used to assess cardiac characteristics by planimetry, as previously described (9). Rats with a moderate-to-large MI were defined as those having an infarct size ≥30% of the myocardium. Those having an LV infarct size ≤25% were defined as those having a sham-to-small MI. Those having an LV infarct size between 25% and 30% were excluded.

Assessment of cardiac fibrosis. This procedure consisted of using samples from both cross-sections cut into 8-μm thick slices and stained with Sirius red F3BA. The collagen network was quantified by computer-assisted image analysis; collagen volume density fraction was determined as previously described (9) by measuring the area of stained tissue within a given field and expressing that area as a proportion of the total area under observation. The collagen-rich border zone of vessels was not included in the calculations. Ten fields were analyzed in the subendocardial layer and ten fields in the subepicardial layer in each LV.

Immunohistochemistry for tumor necrosis factor-alpha (TNF-α). Expression of TNF-α in cardiac tissues was determined on two of the cardiac cross-sections (6 μm). Sections were exposed to goat polyclonal anti-rat TNF-α immunoglobulin-G (IgG) (1:100 dilution) (R&D Systems, Minneapolis, Minnesota). A purified nonspecific goat IgG (1:100 dilution) was used as a primary negative control. The secondary antibodies were biotinylated horse anti-goat IgG (1:400 dilution) (Santa Cruz, Santa Cruz Biotechnology Inc., California). Revelation of bound antibodies was achieved with an avidin/peroxidase complex (Vector Laboratories Inc., Burlingame, California) and counter-stained in Gill's hemotoxylin solution. Positive TNF-α expression was confirmed by a specific brown staining of the cells. Each segment was analyzed with a dedicated 3CCD video microscope adapted to a customized software by an unbiased observer.

Morphology and cytokine mRNA expression group. MORPHOLOGIC PARAMETERS OF HEARTS AT TIME OF INDUCED DEATH. After completing the cardiac hemodynamic measurements, a total of 138 rats were used for biochemical and molecular biologic measurements. All portions of the hearts, as well as the lungs and the kidney, were then weighed individually, frozen in liquid nitrogen and stored at −80°C until used for biochemical investigations. The scarred area was pinned on a paper, and its surface was determined by planimetry (Labtronics Inc., Guelph, Ontario, Canada). Rats with a moderate-to-large MI were defined as those having an LV scar of ≥0.8 cm² of the surface of the scar (9). Those with an LV scar of ≤0.5 cm² were defined as those having a sham-to-small. Those with an LV scar between 0.5 cm² and 0.8 cm² MI were excluded.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of TNF-α, transforming growth factor-beta (TGF-β_1) and interleukin-10 (IL-10) mRNA expression. The RT-PCR experiments were performed on total ribonucleic acid based on a method published by Dukas et al. (10). The following oligonucleotides were utilized as PCR primers: 5′-T ACT GAA CTT CGG GGT GAT TGG TCC-3′; and 5′-CAG CCT TGT CCC TTG AAG AGA ACC-3′; (Clontech, Palo Alto, California) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat TNF-α fragments; 5′-C TTC AGC TCC ACA GAG AAG AAC TGC-3′ and 5′-CAC GAT CAT GTT GGA CAA CTG CTC C-3′ (Clontech) were used as sense and anti-sense primers, respectively, for the amplification of the specific rat TGF-β_1 fragment, and 5′-TG AAG GTC GGT GTC AAC GGA TTT GGC-3′ and 5′-CAT GTA GGC CAT GAG GTC CAC CAC-3′ (Clontech) were used as sense and anti-sense primers, respectively, for the amplification of the specific rat glyceradlehyde-3-phosphate dehydrogenase fragment. The oligonucleotides utilized as PCR primers for IL-10 were purchased from BioSource International (Camarillo, California) and used according to the conditions established by the manufacturer.

Statistical analysis. All data are expressed as means ± SEM. Statistical significance was calculated using a one-way analysis of variance and, when indicated, a post-hoc Bonferroni's test for multiple comparisons. The significance of any differences between two groups was tested using a Student's unpaired t test when appropriated. Only probability values of $p < 0.05$ were accepted as statistically significant. Kaplan-Meier survival curves over the follow-up period were constructed and analyzed by the generalized savage (Mantel-Cox) test.

RESULTS

Survival. Rats in the sham-to-small MI groups all survived until the end of the study (Fig. 1). In the untreated rats, there were 52 sham-to-small MI, and 47 with a moderate-to-large MI, of which 15 died and 32 survived (68% survival). Of the rats receiving omapatrilat 40 mg/kg/day, 27 were sham-to-small MI, and 35 had a moderate-to-large MI, of which 4 died and 31 survived (89% survival, p = 0.01 vs. untreated). Of the rats receiving omapatrilat 80 mg/kg/day, 23 were sham-to-small MI, and 33 had a moderate-to-large MI, of which 5 died and 28 survived (85% survival, p = 0.06 vs. untreated). Of the rats receiving captopril, 29 were sham-to-small MI, and 36 had a moderate-to-large MI, of which 4 died and 32 survived (89% survival, p = 0.03

Table 1. Hemodynamic Measurements at 56 Days After Infarction

		HR (beats/min)	LVSP (mm Hg)	LVEDP (mm Hg)	LV + dP/dt (mm Hg)	RVSP (mm Hg)	RVEDP (mm Hg)	RV + dP/dt (mm Hg)
Sham-to-small MI								
Salin	(n = 52)	242 ± 5	118 ± 3	6 ± 1	6,595 ± 177	25 ± 0	3 ± 0	1,292 ± 39
Omapatrilat 80 mg/kg	(n = 23)	248 ± 5	88 ± 2*	7 ± 1	5,294 ± 193*	25 ± 1	3 ± 0	1,212 ± 46
Omapatrilat 40 mg/kg	(n = 27)	241 ± 5	100 ± 4*	7 ± 1	5,916 ± 183*	24 ± 1	2 ± 0	1,264 ± 34
Captopril 160 mg/kg	(n = 29)	254 ± 6	99 ± 3*	6 ± 1	6,078 ± 164	27 ± 1	3 ± 0	1,283 ± 64
Moderate-to-large MI								
Salin	(n = 29)	254 ± 7	109 ± 3†	20 ± 2†	5,128 ± 213†	41 ± 3†	3 ± 1	1,762 ± 101†
Omapatrilat 80 mg/kg	(n = 28)	245 ± 5	86 ± 2*	14 ± 2*†	4,313 ± 213*†	32 ± 3†	4 ± 1	1,514 ± 106†
Omapatrilat 40 mg/kg	(n = 31)	252 ± 7	92 ± 3*†	13 ± 2*†	4,505 ± 164*†	28 ± 1*†	3 ± 1	1,383 ± 67*
Captopril 160 mg/kg	(n = 32)	256 ± 7	95 ± 3*†	14 ± 2*†	4,855 ± 233†	29 ± 2*	3 ± 1	1,400 ± 72*

Values are given as mean ± SEM. *p < 0.05 vs. untreated; †p < 0.05 vs. sham-to-small MI.

HR = heart rate; LV + dP/dt = left ventricular rate of pressure development; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MI = myocardial infarction; RV + dP/dt = right ventricular rate of pressure development; RVEDP = right ventricular end-diastolic pressure; RVSP = right ventricular systolic pressure.

vs. untreated). In moderate-to-large MI, a mean of 40% to 44% of the LV circumference was infarcted.

Hemodynamic measurements. In sham-to-small MI rats, the various treatment regimens resulted in a decrease in LV systolic pressure (LVSP) and with omapatrilat by a decrease in the maximum rate of pressure rise of the LV (+dP/dt) (Table 1). No other change in hemodynamic parameters was observed.

As compared with sham-to-small MI, untreated moderate-to-large MI rats had a decrease in LVSP and LV +dP/dt and an increase in LV end-diastolic pressure (LVEDP), in RV systolic pressure (RVSP) and RV +dP/dt. Right ventricular end-diastolic pressure did not change. All treatments further decreased LVSP and LVEDP, which, nevertheless, remained higher than in the sham-to-small MI groups. All treatment groups decrease in RVSP and RV +dP/dt as compared with untreated moderate-to-large MI, but this did not reach statistical significance in the 80-mg/kg/day omapatrilat group.

Serum neurohumoral measurements. In sham-to-small MI rats, serum Ang II, ET-1 and catecholamines were similar, regardless of treatment group; ANP was increased in the two omapatrilat groups (from 10 ± 0.5 pg/ml in untreated to 41 ± 3 pg/ml for both omapatrilat, p < 0.05).

In the moderate-to-large MI untreated group, all neuro-

humoral levels increased: ANP from 10 ± 0.5 pg/ml to 41 ± 3 pg/ml, p < 0.05; Ang II from 53 ± 4 pg/ml to 886 ± 72 pg/ml, p < 0.05; ET-1 from 0.7 ± 0.1 to 2.5 ± 0.5 pg/ml, p < 0.05; norepinephrine 54 ± 3 pg/ml to 309 ± 24 pg/ml, p < 0.05; epineprine and dopamine (data not shown). All treatments resulted in a decrease in Ang II (range of 33 to 59 pg/ml, p < 0.05), ET-1 (range of 0.84 to 0.98 pg/ml, p < 0.05) and norepinephrine (range of 102 to 152 pg/ml, p < 0.05). Captopril (35 ± 2 pg/ml) and omapatrilat 40 mg/kg (40 ± 1 pg/ml) did not modify ANP, but omapatrilat 80 mg/kg further increased ANP levels (98 ± 8 pg/ml, p < 0.05 vs. untreated large MI).

Cardiac remodeling and morphologic studies. In sham-to-small MI rats, the three treatment groups resulted in a decrease in LV weight (LVW) to body weight (BW) ratio (2.1 ± 0.3 mg/g to 1.6 to 1.7 ± 0.5 mg/g, p < 0.05) and a decrease in LV circumference and thickness of the intact tissue as compared with the untreated group (Table 2).

The moderate-to-large MI untreated had an increase in LVW/BW (2.4 ± 0.5 mg/g, p < 0.05) and an even greater increase in LV dilation (endocardial and epicardial areas). This was accompanied by an increase in right ventricular weight (RVW)/BW (0.67 ± 0.05 mg/g, p < 0.05), in atrial weight (AW)/BW (0.54 ± 0.05 mg/g, p < 0.05) and in wet lung weight/BW (3.6 ± 0.07 mg/g to 6.7 ± 0.4 mg/g, p <

Table 2. Morphologic Characteristics of Mild LV Cross-Sections

		Infarct Size (%)	Mean Scar Thickness (m)	Epicardial Myocardium Length (mm)	Area Endocardial (mm²)	Area Epicardial (mm²)	Cardiac Fibrosis (%)
Sham-to-small MI							
Salin	(n = 31)	10.52 ± 0.17	0.28 ± 0.00	33.09 ± 0.01	5.34 ± 0.00	9.62 ± 0.00	5 ± 0.00
Omapatrilat 80 mg/kg	(n = 7)	10.68 ± 1.03	0.27 ± 0.00	29.27 ± 0.01*	5.15 ± 0.01	8.23 ± 0.01*	5.5 ± 0.00
Omapatrilat 40 mg/kg	(n = 19)	7.78 ± 0.25	0.28 ± 0.00	29.81 ± 0.01*	4.65 ± 0.00	7.36 ± 0.00*	3.7 ± 0.00
Captopril 160 mg/kg	(n = 15)	10.34 ± 0.36	0.25 ± 0.00	29.53 ± 0.01*	5.01 ± 0.00	8.25 ± 0.01*	3.9 ± 0.00
Moderate-to-large MI							
Salin	(n = 17)	0.39 ± 0.36	0.40 ± 0.00†	25.75 ± 0.02†	7.56 ± 0.00†	11.58 ± 0.01†	11.6 ± 0.01†
Omapatrilat 80 mg/kg	(n = 14)	4.15 ± 0.60	0.52 ± 0.008*†	21.76 ± 0.01*†	6.06 ± 0.00*†	9.04 ± 0.01*	8.3 ± 0.00*†
Omapatrilat 40 mg/kg	(n = 21)	0.61 ± 0.40	0.53 ± 0.00*†	22.05 ± 0.01*†	5.70 ± 0.00*†	8.44 ± 0.01*†	6.6 ± 0.00*
Captopril 160 mg/kg	(n = 20)	2.87 ± 0.32	0.44 ± 0.00†	21.54 ± 0.01*†	5.77 ± 0.01*†	8.69 ± 0.01*	5.8 ± 0.00*

Values are given as mean ± SEM. *p < 0.05 vs. untreated; †p < 0.05 vs. sham-to-small MI.
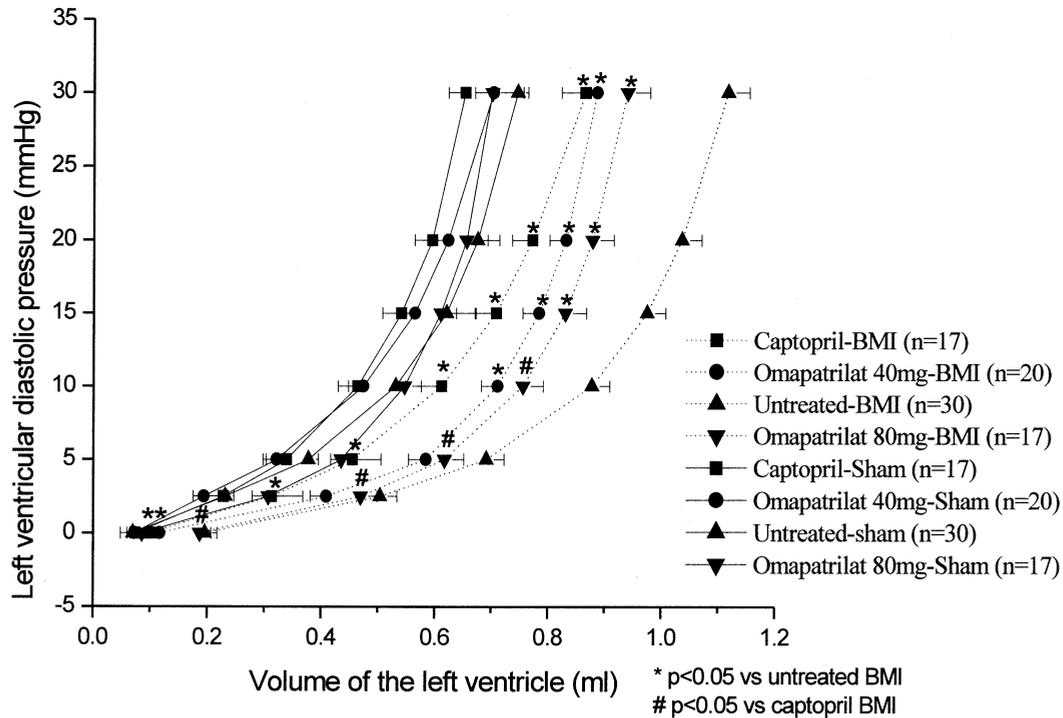LV = left ventricular; MI = myocardial infarction.

Figure 2. Left ventricle pressure-volume relations according to myocardial infarction (MI) size and treatment group. BMI = large MI.

0.05), compatible with lung congestion. All treatments resulted in a decrease in LVW/BW (range of 1.9 to 2.0 ± 0.05 mg/g, p < 0.05) and LV dilation despite similar MI sizes (40% to 44%). This was accompanied by a decrease in RVW (range of 0.40 mg/g to 0.46 ± 0.02 mg/g, p < 0.05), in AW/BW (range of 0.22 to 0.29 ± 0.02 mg/g, p < 0.05) and in lung/BW (range of 4.2 to 4.8 mg/g ± 0.3 mg/g, p < 0.05). However, only RVW/BW and AW/BW returned to sham-to-small MI values. All treatments reduced cardiac fibrosis similarly in moderate-to-large MI hearts. Omapatrilat increased scar thickness more than captopril.

Passive pressure-volume relationships. In sham-to-small MI rats, pressure-volume relationships were similar regardless of treatment group (Fig. 2). As expected, a moderate-to-large MI caused a rightward shift of this relationship as compared with sham-to-small MI. At higher filling pressures (15, 20, 30 mm Hg), all treatments improved this relationship by causing a leftward shift, but not to normal values. However, at lower filling pressures (0, 2.5, 5 and 10 mm Hg), omapatrilat caused less of a leftward shift than captopril, and omapatrilat 80 mg/kg caused a significant rightward shift in the LV pressure-volume curve as compared with captopril, indicating greater ventricular dilation at lower filling pressures despite a similar MI size.

Cytokine expression of the LV. As compared with sham-to-small MI, 56 days after MI the untreated moderate-to-large MI group had no increase in expression of mRNA for the inflammatory cytokine TNF-α, or for the anti-inflammatory cytokine IL-10, but had a significant increase

in the hypertrophic and profibrotic cytokine TGF-β_1 (Fig. 3). Nevertheless, on immunohistochemical staining, TNF-α could be found in the peri-infarct area indicating localized increased expression. Treatment with omapatrilat 40 mg/kg did not modify TNF-α or IL-10 mRNA expression as compared with untreated moderate-to-large MI but normalized TGF-β_1 expression. Captopril also reduced TGF-β_1 and did not modify IL-10 expression, but contrary to omapatrilat, it also reduced TNF-α expression to levels lower than untreated sham-to-small MI and moderate-to-large MI groups.

DISCUSSION

This study indicates that both the ACEI, captopril and the VPI omapatrilat markedly improve post-MI survival, cardiac function and cardiac remodeling in the rat. Despite causing similar reductions in ventricular weights and cardiac fibrosis, the pattern of ventricular remodeling differs between omapatrilat and captopril, the leftward shift in the LV pressure-volume relationship with omapatrilat being less marked than with captopril at lower filling pressures. Both captopril and omapatrilat reduced circulating Ang II, ET-1 and catecholamines, but only omapatrilat modified ANP, increasing it. Both interventions reduced mRNA expression of TGF-β_1; neither effected mRNA expression of IL-10, and only captopril reduced TNF-α. Thus, despite some differences in neurohumoral modulation, ventricular remodeling and effects on cardiac cytokine expression, both
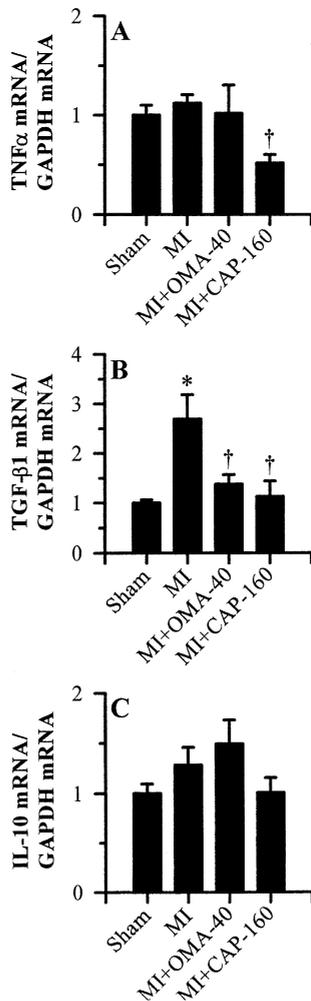
Figure 3. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of tumor necrosis factor-alpha (TNF-α) **(A)**, transforming growth factor-beta (TGF-β_1) **(B)** and interleukin-10 (IL-10) **(C)** messenger ribonucleic acid (mRNA) expression in the left ventricle 56 days after induction of a moderate-to-large myocardial infarction (MI) or after sham-to-small operation. Drug treatments (omapatrilat 40 mg/kg/day [OMA-40] and captopril 160 mg/kg/day [CAP-160]) were started 4 h after coronary artery ligation. Values are means ± SEM of seven to nine animals in each group. Values (Cytokine-to-glyceraldehyde-3-phosphate dehydrogenase [GAPDH] ratio, derived from RT-PCR) are arbitrary scanning units normalized to sham group = 1. *$p < 0.05$ vs. sham-to-small; †$p < 0.05$ vs. MI.

captopril and omapatrilat had similar effects on cardiac function and survival.

Effects on neurohormones. The doses of omapatrilat chosen increased circulating ANP, and, in previous studies, have been shown to inhibit the pressor response to Ang II and to increase urinary excretion of ANP in the cardiomyopathic hamster (6) and pacing overdrive dog (7) and to reduce metabolism of exogenous bradykinin more than either ACE or NEP inhibition alone (8). In this study, both captopril and omapatrilat significantly reduced circulating Ang II in MI rats, consistent with inhibition of ACE and/or hemodynamic improvement. Also, consistent with hemodynamic improvement, all groups decreased ET-1 and

norepinephrine. These findings suggest that, in this setting, concerns regarding deleterious effects of NEP inhibition on ET-1 and of BK on catecholamine release did not materialize (11). However, these results need to be viewed with some caution, as circulating neurohumoral values are only imperfect reflections of what happens at the tissue level.

Effects on survival. Both captopril and omapatrilat resulted in a significant improvement in survival. This occurred despite differences in neurohumoral modulation, in ventricular remodeling, and cardiac cytokine expression. This suggests that the common beneficial effects that they exert on neurohumoral modulation, on cardiac hemodynamics, on ventricular remodeling and on cardiac cytokine expression are more important than the differences or that the differences between the two drugs balance themselves off. Due to the relatively short follow-up period of the study, we cannot, however, rule out that one of these differences would eventually result in improved survival.

Hemodynamic effects. Both captopril and omapatrilat improved cardiac hemodynamics similarly. This was true for both the LV and RV and at both doses of omapatrilat used. In addition, both medications decreased lung to BW ratio, suggesting that both agents also reduced pulmonary congestion. Taken together with the survival data, these findings suggest that omapatrilat does not exert a superior effect to that of the ACEI captopril. Considering the cardioprotective effects demonstrated for bradykinin in experimental studies (1,2) and the beneficial effects of an ANP infusion in patients with an acute anterior MI (4), these results were somewhat surprising. They suggest that increases in BK above those caused by ACEIs are not useful and that the levels of ANP achieved by VPIs after MI are inadequate to have significant effects in this setting in the rat.

Effects on cardiac remodeling. Both captopril and omapatrilat reduced LVW and ventricular dimensions similarly in the sham-to-small MI groups and in the moderate-to-large MI groups. Consistent with their hemodynamic effects, both drugs also reduced RVW, AW and lung weight in the moderate-to-large MI groups. Interestingly, despite reducing cardiac fibrosis, omapatrilat resulted in an increase in scar thickness that appeared to be superior to that of captopril. The reason for this difference is speculative but could result from increased peri-MI inflammatory response in the area of the scar. Increased inflammation could help prevent early scar expansion by stabilizing the scar, and the degree of inflammatory response is considered one of the determinants of scar healing (1). Kinin-B$_2$ receptors have been found to be upregulated in both the infarcted and noninfarcted areas of the heart (12) and may be important for scar stabilization and initiating repair. In a previous study, we have shown that omapatrilat reduces BK metabolism in the scar more than ACEIs (8).

Captopril reduced post-MI ventricular dilation, that is, change in volume relative to a change in pressure. Although omapatrilat caused a similar attenuation in this rightward shift at higher filling pressures, at lower filling pressures (0,

2.5, 5 and 10 mm Hg) this attenuation was significantly less than with captopril, particularly in the omapatrilat 80 mg/kg group. Because the scar in the omapatrilat group was, if anything, thicker, we are at a loss to explain these findings. We also cannot explain the equalization of volumes at higher filling pressures, except to speculate that differences in the makeup of the fibrotic tissue between captopril and omapatrilat exist. With omapatrilat 80 mg/kg, the steeper pressure-volume relationship at higher filling pressures may have resulted from greater interstitial fibrosis.

Effects on cardiac cytokine mRNA expression. The cardiac expression of several cytokines were measured because of a possible omapatrilat-induced BK-induced potentiation of their expression. In situations of injury, BK is known to potentiate the inflammatory response (11). Both captopril and omapatrilat reduced the growth- and fibrosis-promoting cytokine TGF-β_1, a finding compatible with the reduction in LVW and cardiac fibrosis found in hearts treated with both drugs. Both drugs had no effect on the expression of the anti-inflammatory IL-10, but only captopril reduced cardiac expression of the proinflammatory cytokine TNF-α. We, as others before us, found that cardiac expression of TNF-α in the noninfarcted area returned towards baseline levels several weeks after MI, but, as others before us (13,14), we found that a persistent excess expression of TNF-α occurs in the peri-MI area. By reducing the expression of TNF-α, captopril could prevent progressive deterioration of LV function after MI and result in improved long-term survival.

Conclusions. This study indicates that both omapatrilat and captopril markedly improve post-MI survival, cardiac function and cardiac remodeling in the rat. It would appear that the addition of NEP inhibition to ACE inhibition does not result in significant further benefit.

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