Effects of captopril and omapatrilat on early post-myocardial infarction survival and cardiac hemodynamics in rats: interaction with cardiac cytokine expression

Charles Blais, Jr., Nathalie Lapointe, Jean-Lucien Rouleau, Robert Clément, Dimcho R. Bachvarov, and Albert Adam

Abstract: The purpose of this study was to evaluate and compare the effects of simultaneous angiotensin-converting enzyme (ACE) and neutral endopeptidase 24.11 (NEP) inhibition by the vasopeptidase inhibitor omapatrilat (10 and 40 mg·kg⁻¹·day⁻¹) with those of the selective ACE inhibitor captopril (160 mg·kg⁻¹·day⁻¹) on survival, cardiac hemodynamics, and cytokine mRNA expression in left ventricular (LV) tissues 4 days after myocardial infarction (MI) in rats. The effects of the co-administration of both B₁ and B₂ kinin receptor antagonists (2.5 mg·kg⁻¹·day⁻¹ each) with and without omapatrilat were also evaluated to assess the role of bradykinin (BK) during this post-MI period. Both omapatrilat and captopril treatments improve early (4 days) post-MI survival when started 4 h post-MI. The use of kinin receptor antagonists had no significant effect on survival in untreated MI rats and omapatrilat-treated MI rats. This improvement in survival with omapatrilat and captopril is accompanied by a reduced LV end-diastolic pressure (LVEDP) and pulmonary congestion. The use of kinin receptor antagonists had little effect on cardiac hemodynamics or morphologic measurements. Acute MI significantly increased the expression of cardiac cytokines (TNF-α, TGF-β₁, and IL-10). Captopril significantly attenuated this activation, while omapatrilat had variable effects: sometimes increasing but generally not changing activation depending on the cytokine measured and the dose of omapatrilat used. The co-administration of both kinin receptor antagonists attenuates the increase in expression of cardiac TNF-α and TGF-β₁ after omapatrilat treatment. Taken together, these results would suggest that despite very marked differences in the way these drugs modified the expression of cardiac cytokines, both omapatrilat and captopril improved early (4 days) post-MI survival and cardiac function to a similar extent.

Key words: ACE inhibitor, cytokines, kinins, myocardial infarction, vasopeptidase inhibitor.


C. Blais, Jr., and A. Adam. Faculté de Pharmacie, Université de Montréal, 2900, boul. Édouard-Montpetit, C.P. 6128, Succursale Centre-ville, Montréal, QC H3C 3J7, Canada.

N. Lapointe and J.-L. Rouleau. Division of Cardiology, University Health Network, Toronto General Hospital, Toronto, ON M5G 2C4, Canada.

R. Clément. Institut de Cardiologie de Montréal, Montréal, QC H1T 1C8, Canada.

D.R. Bachvarov. Centre Hospitalier Universitaire de Québec, Centre de Recherche du Pavillon l’Hôtel-Dieu de Québec, Québec, QC G1R 2J6, Canada.

Corresponding author (e-mail: albert.adam@umontreal.ca).

Introduction

Angiotensin-converting enzyme (ACE, EC 3.4.25.1) inhibition has been shown to improve the survival of patients when started early (<24 h) after myocardial infarction (MI), particularly in the presence of left ventricular dysfunction (Brown and Vaughan 1998; Yusuf et al. 2000). In MI patients, the majority of the benefit is noted during the first week post-MI. Since the renin-angiotensin system is activated in the setting of an MI, it is attractive to postulate that the beneficial effects of ACE inhibitors are only attributable to a decreased synthesis of angiotensin II, a potent vasoconstrictive and salt-retentive peptide that stimulates myocardial hypertrophy and plays a role in growth of the cardiac interstitium (Brown and Vaughan 1998). However, ACE, also called kininase II, acts as a potent kinin-degrading enzyme in plasma and tissues. Thus, there is mounting evidence that at least part of the beneficial effect of ACE inhibitors are the result of the inhibition of kinin metabolism (Linz et al. 1995; Blais et al. 2000b). In acute MI, bradykinin (BK) can exert beneficial effects by systemic and coronary vasodilatation, thereby improving metabolic balance, lowering myocardial oxygen consumption, and potentially reducing infarct size (Linz et al. 1995). BK can also improve cardiac function through diuretic, natriuretic, anti-hypertrophic, anti-proliferative, anti-thrombotic, and fibrolytic effects due to the release of prostacyclin, nitric oxide, and endothelium-derived hyperpolarizing factor (Levinsky 1979; Moncada et al. 1991; Linz et al. 1995; Blais et al. 2000b). Thus, BK may help to counteract some of the detrimental effects of renin-angiotensin activation.

Early post-MI is also characterized by activation of an inflammatory process in which the pro-inflammatory properties of BK may play an important contributory role. As part of this inflammatory process, there is increased expression of several cytokines (Thompson et al. 1988; Herskowitz et al. 1995; Ono et al. 1998). Indeed, increased plasma levels as well as local myocardial production of several cytokines such as tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-6, and transforming growth factor β1 (TGF-β1) have been observed in patients early post-MI (Maury and Teppo 1989; Ikeda et al. 1992; Basaran et al. 1993). There is evidence that cytokines have been implicated in the pathogenesis of myocardial dysfunction and cardiomyocyte death in MI and congestive heart failure (CHF) (Pulkki 1997; Blum and Miller 1998; Meldrum 1998). Moreover, several cytokines have been reported to regulate cardiac myocyte growth, contractile protein synthesis, fibroblast proliferation, and extracellular matrix gene expression (Meldrum 1998; Sasayama et al. 1999; Lijnen et al. 2000). These observations suggest that cytokines may be important modulators in the post-MI process.

Neutral endopeptidase 24.11 (NEP, EC 3.4.24.11) is another important enzyme involved in the degradation of kinins and is the major enzyme inactivating natriuretic peptides (ANP, BNP, and CNP) (Erdös and Skidgel 1989). Inhibition of NEP potentiates the vasodilator, diuretic, and natriuretic effects of these two peptidergic systems and has beneficial effects in animal models of heart failure (Seymour et al. 1993; Rademaker et al. 1996; Willenbrock et al. 1996). It may thus be postulated that the simultaneous inhibition of ACE and NEP may be superior to ACE inhibition alone post-MI. A new class of drugs has been developed, the vasopeptidase inhibitors, which are single molecules that simultaneously inhibit both ACE and NEP with similar nanomolar inhibitory constants. These vasopeptidase inhibitors were shown to prevent the development of cardiac hypertrophy and reduce cardiac preload in rats with MI (Bralet et al. 1994; Marie et al. 1995). They display hemodynamic effects that are superior to those induced by selective inhibition of ACE or NEP in cardiomyopathic hamsters (Trippodo et al. 1995) and, in the pacing-induced left ventricular (LV) dysfunction dog, they reduce the degree of LV dilation and improve isolated myocyte contractile function, showing no apparent effect on LV-pump function (Thomas et al. 1998). Omapatrilat is the clinically most advanced vasopeptidase inhibitor and is now being investigated in clinical trials for use in hypertension and congestive heart failure, and its use early post-MI is also being considered.

In previous studies, we demonstrated that the vasopeptidase inhibitor omapatrilat reduces the metabolism of endogenous BK more than ACE or NEP inhibition in rat and human hearts (Raut et al. 1999; Blais et al. 2000a). Because endogenous BK levels are cardioprotective in the acute MI setting, we hypothesized that omapatrilat could have greater cardioprotective effects than ACE inhibitors in the early post-MI setting, and that this effect could be related to an increase in endogenous kinin cardiac levels. Alternatively, the extra protection of BK by simultaneous ACE and NEP inhibition could excessively stimulate the post-MI inflammatory response and cardiac cytokine expression and lead to detrimental effects.

The purpose of this study was to evaluate and compare the effects of simultaneous ACE and NEP inhibition by the vasopeptidase inhibitor omapatrilat with those of the selective ACE inhibitor captopril on survival, cardiac hemodynamics, and cytokine mRNA expression in LV cardiac tissues 4 days after MI in rats. The effects of the co-administration of both B1 and B2 kinin receptor antagonists...
with and without omapatrilat were also evaluated to assess the role of BK during this post-MI period.

**Materials and methods**

**Drugs, peptides, and reagents**

The ACE inhibitor captopril (IC$_{50}$ = 23 nM) and the vasopeptidase inhibitor omapatrilat, which acts by combined inhibition of ACE (IC$_{50}$ = 5 nM) and NEP (IC$_{50}$ = 9 nM), were provided for research purposes by Bristol-Myers Squibb (Princeton, N.J.). The B$_2$ kinin receptor antagonist icatibant (HOE 140, D-Arg-[Hyp$^3$,Thi$^5$,D-Tic$^6$,Oic$^8$]BK; Wirth et al. 1991) was a generous gift from Dr. K.J. Wirth (Hoechst AG, Frankfurt, Germany). The B$_1$ kinin receptor antagonist R-715, Ac-Lys-[D-$\beta$-Na$_3$,Ile$_8$]Des-Arg$_9$-BK (Gobeil et al. 1999), was obtained from Dr. D. Regoli (Université de Sherbrooke, Sherbrooke, Que.). Ketamine hydrochloride was obtained from Rogar/STB (Montreal, Que.), xylazine was from Bayer Canada (Etobicoke, Ont.), and buprenorphine HCl was from Reckitt Colman Pharmaceuticals (Richmond, Va.). Halothane was manufactured by Halocarbon Laboratories (Riveredde, N.J.). All other chemicals of analytic grade were obtained from Fisher Scientific (Montreal, Que.).

**Myocardial infarction**

An MI was induced in 175 male Wistar rats (Charles River, St. Constant, Que.) weighing 200–250 g by ligation of the left anterior descending coronary artery (LAD) as described earlier by Pfeffer et al. (1979). Animals were given 5–7 days to adjust to their new environment. 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, Que.). Animals were anesthetized with 3% halothane. During the surgery, they were artificially ventilated with humidified room air supplemented with oxygen, and the halothane concentration was gradually decreased to 1%. A Harvard rodent ventilator (Harvard Apparatus, South Natick, Mass.) set at 2 mL, 70 strokes/min, and a Fluotec 3 halothane vaporizer (Cyprane) were used for this procedure. A left thoracotomy was performed via the fourth intercostal space, and the lungs were retracted to expose the heart. After the pericardium was opened, the LAD was ligated ~2 mm from its origin using a 4-0 silk (Ethicon, Johnson and Johnson, U.S.A.); ligation was deemed successful when the anterior wall of the LV turned pale. The chest was closed with a 2-0 silk (Ethicon, Johnson and Johnson, U.S.A.) and the incision was closed with a Mikron wound clip applicator (Clay Adams, Parsippany, N.J.) after the chest was gently pressed to expel air from the cavity to avoid a pneumothorax. Once awake, the rats were injected intramuscularly with buprenorphine HCl 0.01 mg·kg$^{-1}$ to reduce the pain during recovery. The sham ligation group underwent a similar procedure except that the suture was not tightened around the coronary artery. The rats were housed for 4 days in clear plastic cages in an air-conditioned room with a 12 h photoperiod with free access to normal crush laboratory rat chow and tap water. Of these 175 rats, 44 died 4 h post-operation or during the 4 day follow-up period. Those dying between 4 h and 24 h post-infarction were assumed to have had a large MI. Those dying later than 24 h post-coronary lig- gation but prior to hemodynamic monitoring had morphologic assessment for classification of MI size but were not used for other measurements except for survival. The survivors were randomized into different groups.

**Drug randomization**

At the time of the MI, rats were first divided to receive the combination of both B$_2$ (icatibant) and B$_1$ (R-715) kinin receptor antagonists (2.5 mg·kg$^{-1}$·day$^{-1}$ each) or not. B$_1$ and B$_2$ antagonists were given together because, with inflammation such as may occur in this model, B$_1$ receptors may be expressed and exert effects similar to those of B$_2$ receptors. Those randomized to receive the B$_2$ and B$_1$ receptor antagonists received these agents dissolved in sterile saline via continuous infusion from a micro-osmotic pump (Alzet 1007D, Alza, Palo Alto, Calif.) that was inserted subcutaneously in the back of the animal during the surgery for the MI (Blais et al. 1997; Gobeil et al. 1999). Four hours after MI, rats were again randomly divided into six groups according to their therapeutic intervention. After randomization (4 h post-MI), rats had an intraperitoneal injection of their assigned medication. This procedure was repeated the next morning to assure adequate levels of medications post-MI. Thereafter rats received their medications with the food. The drugs were mixed with normal powdered chow, and total chow intake was monitored every day. A first group received intraperitoneal injections of saline followed by normal food (untreated group, n = 21). A second group received intraperitoneal injections of 4 mg·kg$^{-1}$ omapatrilat, and 40 mg·kg$^{-1}$·day$^{-1}$ in food thereafter (n = 20). A third group received intraperitoneal injections of 1 mg·kg$^{-1}$ omapatrilat, and 10 mg·kg$^{-1}$·day$^{-1}$ in food thereafter (n = 17). A fourth group received intraperitoneal injections of 16 mg·kg$^{-1}$ captopril, and 160 mg·kg$^{-1}$·day$^{-1}$ in food thereafter (n = 27). A fifth group received both B$_2$ and B$_1$ kinin receptor antagonists (icatibant and R-715, respectively) and intraperitoneal injections of 4 mg·kg$^{-1}$ omapatrilat, and 40 mg·kg$^{-1}$·day$^{-1}$ in food thereafter (n = 31). A sixth group received both B$_2$ and B$_1$ kinin receptor antagonists (icatibant and R-715, respectively) and intraperitoneal injections of normal saline solution, and normal food thereafter (n = 15). The dosages of captopril and omapatrilat were based on previous studies (Pfeffer et al. 1985a, 1985b; Trippodo et al. 1999; d’Uscio et al. 2001).

**Cardiac hemodynamic measurements**

After 4 days of therapy, the rats were anesthetized with an intramuscular injection of a ketamine (50 mg·kg$^{-1}$) and xylazine (10 mg·kg$^{-1}$) mixture. The trachea was intubated by a non-invasive method via the mouth and mechanically ventilated with room air supplemented with low-flow oxygen with a small-rat ventilator (Harvard Apparatus). An electrocardiogram (ECG) was performed, and the left and right ventricular pressures were measured by a Millar micro-tip catheter transducer (Millar Instruments, Houston, Tex.) with a pressure sensor at the tip. The catheter was inserted into the jugular vein and carotid artery and advanced to the right and left ventricles, respectively. The maximum rate of pressure rise (+dP/dt) and decline (–dP/dt) of both ventricles were also measured. The ECG and pressures were recorded on a Gould 2600S recorder (Gould, Cleveland, Ohio). Be-
cause of equipment problems or death during the procedure, hemodynamic measurements could not be performed in some animals: 2 rats of group 1, 1 of group 2, 1 of group 3, 1 of group 4, 1 of group 5, and 1 of group 6. There was no difference in cardiac hemodynamics between the untreated groups with or without the BK receptor antagonists, as well as with both doses of omapatrilat, so these groups were combined.

Cardiac morphologic assessment
Once the hemodynamic measurements were obtained, the rats were deeply anesthetized and killed by cardio-pulmonary excision. The heart was rapidly rinsed in saline solution, and dissected into right and left atria, right ventricle, left ventricle, septum, and scar. The scarred area was pinned on a paper and its surface was determined by planimetry (Labtronics Inc., Guelph, Ont.). All portions of the hearts as well as the lungs were then weighed individually, frozen in liquid nitrogen, and stored at –80°C until being used for biochemical investigations.

Animals were not classified according to MI size until the end of the study at which time they were separated into sham to small MI or medium to large MI groups. Rats with a M of each of the TNF-α or TGF-β1 primers with 0.027 μM or 0.04 μM of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers (needed for the amplification of an internal standard), respectively, and 2.5 U of Taq polymerase (AmpliTaq DNA Polymerase, Perkin-Elmer). 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' (Clonetech, Palo Alto, Calif.) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat TNF-α fragment; 5'-C TTC AGC TCC ACA GAG AAG AAC TGC-3' and 5'-CAC GAT CAT GTT GGA CAA C TG CTC C-3' (Clonetech) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat TGF-β1 fragment and 5'-GAA GTG TGT GTC AAC GGA TTT GGC-3' and 5'-CAT GAT GGC CAT GAG GTC CAC CAC-3' (Clonetech) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat GAPDH fragment. The oligonucleotides were used according to the conditions established by the manufacturer. The samples were denatured initially for 4 min at 94°C and then submitted to 35 cycles of PCR for TNF-α and 27 cycles of PCR for TGF-β1 (45 s at 94°C, 45 s at 60°C, and 2 min at 72°C) and a final 7-min elongation step at 72°C in a PTC-200 DNA Engine thermal cycler (MJ Research, Inc., Watertown, Mass.). The number of cycles was chosen to keep the PCR-amplified DNA in the exponential phase of amplification. One-fifth of each of the PCR reactions was loaded on a 1.8% agarose gel in 1× Tris-borate–EDTA buffer and 0.5 μg·mL−1 ethidium bromide and electrophoresed at 100 V for approximately 1.5 h. The 1-kb DNA ladder (Gibco-BRL) was used to determine the molecular weight of each PCR fragment. The resulting photographs were scanned with a ScanJet 5100C scanner (Hewlett-Packard, Palo Alto, Calif.) and analyzed with 1D Main densitometry software (AAB Software, Dayton, Ohio).

RT-PCR analysis of the IL-10 mRNA expression
The IL-10 mRNA expression was evaluated in the probes studied by a duplex RT-PCR approach (Dukas et al. 1993) using the Ready-to-Go RT-PCR beads (Amersham Pharmacia Biotech) as indicated by the manufacturer. Each RT-PCR reaction (50 μL final volume) contained 4 μg of total RNA, 20 ng of oligo dT12 primer, 100 ng each of the IL-10 primers, and 10 ng each of the GAPDH primers (needed for the amplification of an internal standard, see above). The oligonucleotides utilized as PCR primers for IL-10 were purchased from BioSource International (Camarillo, Calif.) and used according to the conditions established by the manufacturer. The samples were incubated initially for 30 min at 42°C, followed by a denaturation step for 4 min at 95°C, and were then submitted to 30 cycles of PCR (1 min at 94°C, 1 min at 95°C, and 1.5 min at 60°C) for amplification of an internal standard), respectively, and 2.5 U of Taq polymerase (AmpliTaq DNA Polymerase, Perkin-Elmer). 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' (Clonetech, Palo Alto, Calif.) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat TNF-α fragment; 5'-C TTC AGC TCC ACA GAG AAG AAC TGC-3' and 5'-CAC GAT CAT GTT GGA CAA C TG CTC C-3' (Clonetech) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat TGF-β1 fragment and 5'-GAA GTG TGT GTC AAC GGA TTT GGC-3' and 5'-CAT GAT GGC CAT GAG GTC CAC CAC-3' (Clonetech) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat GAPDH fragment. The oligonucleotides were used according to the conditions established by the manufacturer. The samples were denatured initially for 4 min at 94°C and then submitted to 35 cycles of PCR for TNF-α and 27 cycles of PCR for TGF-β1 (45 s at 94°C, 45 s at 60°C, and 2 min at 72°C) and a final 7-min elongation step at 72°C in a PTC-200 DNA Engine thermal cycler (MJ Research, Inc., Watertown, Mass.). The number of cycles was chosen to keep the PCR-amplified DNA in the exponential phase of amplification. One-fifth of each of the PCR reactions was loaded on a 1.8% agarose gel in 1× Tris-borate–EDTA buffer and 0.5 μg·mL−1 ethidium bromide and electrophoresed at 100 V for approximately 1.5 h. The 1-kb DNA ladder (Gibco-BRL) was used to determine the molecular weight of each PCR fragment. The resulting photographs were scanned with a ScanJet 5100C scanner (Hewlett-Packard, Palo Alto, Calif.) and analyzed with 1D Main densitometry software (AAB Software, Dayton, Ohio).

RT-PCR analysis of the IL-10 mRNA expression
The IL-10 mRNA expression was evaluated in the probes studied by a duplex RT-PCR approach (Dukas et al. 1993) using the Ready-to-Go RT-PCR beads (Amersham Pharmacia Biotech) as indicated by the manufacturer. Each RT-PCR reaction (50 μL final volume) contained 4 μg of total RNA, 20 ng of oligo dT12 primer, 100 ng each of the IL-10 primers, and 10 ng each of the GAPDH primers (needed for the amplification of an internal standard, see above). The oligonucleotides utilized as PCR primers for IL-10 were purchased from BioSource International (Camarillo, Calif.) and used according to the conditions established by the manufacturer. The samples were incubated initially for 30 min at 42°C, followed by a denaturation step for 4 min at 95°C, and were then submitted to 30 cycles of PCR (1 min at 94°C, 1 min at 95°C, and 1.5 min at 60°C) for amplification of an internal standard), respectively, and 2.5 U of Taq polymerase (AmpliTaq DNA Polymerase, Perkin-Elmer). 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' (Clonetech, Palo Alto, Calif.) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat TNF-α fragment; 5'-C TTC AGC TCC ACA GAG AAG AAC TGC-3' and 5'-CAC GAT CAT GTT GGA CAA C TG CTC C-3' (Clonetech) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat TGF-β1 fragment and 5'-GAA GTG TGT GTC AAC GGA TTT GGC-3' and 5'-CAT GAT GGC CAT GAG GTC CAC CAC-3' (Clonetech) were used as sense and anti-sense primers, respectively, for the amplification of a specific rat GAPDH fragment. The oligonucleotides were used according to the conditions established by the manufacturer. The samples were denatured initially for 4 min at 94°C and then submitted to 35 cycles of PCR for TNF-α and 27 cycles of PCR for TGF-β1 (45 s at 94°C, 45 s at 60°C, and 2 min at 72°C) and a final 7-min elongation step at 72°C in a PTC-200 DNA Engine thermal cycler (MJ Research, Inc., Watertown, Mass.). The number of cycles was chosen to keep the PCR-amplified DNA in the exponential phase of amplification. One-fifth of each of the PCR reactions was loaded on a 1.8% agarose gel in 1× Tris-borate–EDTA buffer and 0.5 μg·mL−1 ethidium bromide and electrophoresed at 100 V for approximately 1.5 h. The 1-kb DNA ladder (Gibco-BRL) was used to determine the molecular weight of each PCR fragment. The resulting photographs were scanned with a ScanJet 5100C scanner (Hewlett-Packard, Palo Alto, Calif.) and analyzed with 1D Main densitometry software (AAB Software, Dayton, Ohio).

RT-PCR analysis of the IL-10 mRNA expression
The IL-10 mRNA expression was evaluated in the probes studied by a duplex RT-PCR approach (Dukas et al. 1993) using the Ready-to-Go RT-PCR beads (Amersham Pharmacia Biotech) as indicated by the manufacturer. Each RT-PCR reaction (50 μL final volume) contained 4 μg of total RNA, 20 ng of oligo dT12 primer, 100 ng each of the IL-10 primers, and 10 ng each of the GAPDH primers (needed for the amplification of an internal standard, see above). The oligonucleotides utilized as PCR primers for IL-10 were purchased from BioSource International (Camarillo, Calif.) and used according to the conditions established by the manufacturer. The samples were incubated initially for 30 min at 42°C, followed by a denaturation step for 4 min at 95°C, and were then submitted to 30 cycles of PCR (1 min at 94°C,
1 min at 60°C, 2 min at 72°C) with a 10 min final elongation step at 72°C. Twenty-five microlitres of each RT-PCR reaction were run on a 1% agarose gel in 1× TBE buffer and were then transferred to Hybond-N nylon membranes (Amersham Pharmacia Biotech). The membranes were prehybridized for 1 h at 65°C in a buffer containing 6× SSC, 5× Denhardt’s solution, 0.5% SDS, 100 μg·mL−1 salmon sperm DNA; then 10⁶ cpm/mL of IL-10 random-primed 32P-labelled probe was added and hybridization was carried for 8–16 h at 65°C. The membranes were then stripped and rehybridized with a rat GAPDH probe used as an internal standard. The resulting autoradiograms were scanned with a ScanJet 6 (Hewlett-Packard) and analyzed with the 1D Main densitometry software (AAB Software).

Statistical analysis
All data are expressed as means ± SE for n values. Statistical significance of differences was calculated using one-way analysis of variance and, when indicated, a post hoc Tukey’s test for multiple comparisons. 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
The sham-operated controls all survived until the end of the study. Of the untreated rats, 9 were sham and 28 had an MI, of which 16 died and 12 survived (43% survival). Of the untreated rats that received B1 and B2 receptor antagonists (BK antagonists), 6 were sham and 19 had an MI, of which 10 died and 9 survived (47% survival). Since survival rates were the same in the two untreated groups, they were considered together (45% survival). The rats with an MI that received either of the doses of omapatrilat had a similar survival (82% and 83%) and thus these two groups were considered together. Of the rats receiving omapatrilat, 18 were sham and 23 had an MI, of which 4 died and 19 survived (83% survival, P < 0.05 versus untreated MI). Of the rats receiving captopril, 13 were sham and 18 had an MI, of which 4 died and 14 survived (78% survival, P < 0.05 versus untreated MI).

Morphologic parameters
By definition, scar surface as well as the scar weight to body weight (SW/BW) ratio were significantly increased in hearts with an MI as compared with sham-operated rats. The SW/BW ratios were similar in all MI groups. The MI as well as the various treatment regimens resulted in no other significant changes in total cardiac or individual cardiac chamber weights. There was, however, an increase in lung weight to body weight (LW/BW) ratio in the untreated MI rats as compared with their sham counterparts, suggesting greater pulmonary congestion. This increase was not present in the various MI treatment groups, which is consistent with a decrease in pulmonary congestion. This normalization was particularly evident in the omapatrilat group without the BK antagonist and in the captopril group.

Duplex RT-PCR analysis of cytokine mRNA expression in MI hearts
The TNF-α, TGF-β1, and IL-10 mRNA expression levels in non-infarcted LV tissues from MI hearts were determined using a semiquantitative RT-PCR assay. For these cytokines, a baseline mRNA expression was detected in LV tissues.

TNF-α
The TNF-α mRNA expression was significantly increased by 66% (P < 0.01) in the untreated MI group compared with the sham-operated rats. In both MI groups treated with
Table 1. Cardiac hemodynamics 4 days postinfarction.

<table>
<thead>
<tr>
<th>Heart rate (beats/min)</th>
<th>LV SP (mmHg)</th>
<th>LVEDP (mmHg)</th>
<th>LV+dP/dt (mmHg/s)</th>
<th>LV–dP/dt (mmHg/s)</th>
<th>RV SP (mmHg)</th>
<th>RV+dP/dt (mmHg/s)</th>
<th>RV–dP/dt (mmHg/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated ± BK</td>
<td>109.4 ± 4.7</td>
<td>115.5 ± 5.3</td>
<td>101.7 ± 1.2</td>
<td>133.7 ± 5.8</td>
<td>354 ± 23.2</td>
<td>31.5 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
<td>Omapatrilat 10 mg·kg–1·day–1</td>
<td>107.2 ± 5.6</td>
<td>112.6 ± 5.3</td>
<td>100.5 ± 1.2</td>
<td>144.3 ± 5.8</td>
<td>362 ± 24.2</td>
<td>32.1 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
<td>Captopril 10 mg·kg–1·day–1</td>
<td>106.3 ± 5.4</td>
<td>109.5 ± 5.3</td>
<td>99.8 ± 1.2</td>
<td>142.2 ± 5.8</td>
<td>357 ± 23.2</td>
<td>31.5 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
<td>Omapatrilat + BK</td>
<td>107.2 ± 5.6</td>
<td>112.6 ± 5.3</td>
<td>100.5 ± 1.2</td>
<td>144.3 ± 5.8</td>
<td>362 ± 24.2</td>
<td>32.1 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated ± BK</td>
<td>109.4 ± 4.7</td>
<td>115.5 ± 5.3</td>
<td>101.7 ± 1.2</td>
<td>133.7 ± 5.8</td>
<td>354 ± 23.2</td>
<td>31.5 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
<td>Omapatrilat 10 mg·kg–1·day–1</td>
<td>107.2 ± 5.6</td>
<td>112.6 ± 5.3</td>
<td>100.5 ± 1.2</td>
<td>144.3 ± 5.8</td>
<td>362 ± 24.2</td>
<td>32.1 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
<td>Captopril 10 mg·kg–1·day–1</td>
<td>106.3 ± 5.4</td>
<td>109.5 ± 5.3</td>
<td>99.8 ± 1.2</td>
<td>142.2 ± 5.8</td>
<td>357 ± 23.2</td>
<td>31.5 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
<tr>
<td>Omapatrilat + BK</td>
<td>107.2 ± 5.6</td>
<td>112.6 ± 5.3</td>
<td>100.5 ± 1.2</td>
<td>144.3 ± 5.8</td>
<td>362 ± 24.2</td>
<td>32.1 ± 4.1</td>
<td>24.1 ± 1.2</td>
</tr>
</tbody>
</table>

Note: Values are given as mean ± SE. HR, heart rate; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LV+dP/dt, left ventricular rate of pressure development; LV–dP/dt, left ventricular rate of pressure decline; RVSP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure; RV+dP/dt, right ventricular rate of pressure development; RV–dP/dt, right ventricular rate of pressure decline.

**P < 0.05 vs. untreated ± BK antagonists.**

| Table 1. Cardiac hemodynamics 4 days postinfarction. |

Omapatrilat (10 and 40 mg·kg–1·day–1), the TNF-α mRNA expression was not significantly different from that of the untreated MI group. The TNF-α mRNA expression in MI captopril-treated rats was not significantly increased in comparison with the sham-operated rats, but was also not different from that of the untreated MI group. However, a significant difference was observed between MI groups treated with 40 mg·kg–1·day–1 omapatrilat- and captopril-treated rats (P < 0.01). The co-administration of both kinin receptor antagonists to the untreated MI and 40 mg·kg–1·day–1 omapatrilat-treated rats decreased the TNF-α mRNA expression, as compared with untreated MI (P < 0.05) and omapatrilat (P < 0.001) treated groups.

**TGF-β1**

In the untreated MI rats, the TGF-β1 mRNA expression was significantly increased by 61% (P < 0.05) when compared with the sham-operated rats. In both MI groups treated with omapatrilat 10 mg·kg–1·day–1 and captopril, the TGF-β1 mRNA expression was not significantly different from the untreated MI group, but was also not different from the sham-operated group. However, the TGF-β1 mRNA expression was significantly increased in the MI group treated with 40 mg·kg–1·day–1 omapatrilat, as compared with the sham-operated rats (P < 0.001), the untreated MI group (P < 0.01), the MI 10 mg·kg–1·day–1 omapatrilat-treated rats (P < 0.001), and the MI captopril-treated group (P < 0.001). The co-administration of both icatibant and R-715 to the untreated MI rats did not modify the TGF-β1 mRNA expression, but decreased significantly the TGF-β1 mRNA expression in the 40 mg·kg–1·day–1 omapatrilat treated rats (P < 0.05), as compared with their respective untreated MI and omapatrilat-treated groups.

**IL-10**

The IL-10 mRNA expression was increased by 113% in the untreated MI group, by 121% in the MI 10 mg·kg–1·day–1 omapatrilat treated group, and by 123% in the MI 40 mg·kg–1·day–1 omapatrilat-treated rats. However, these increased expression values did not reach statistical significance (P = 0.094, P = 0.059, and P = 0.054, respectively) despite a significant ANOVA (F[6,35] = 3.028, P < 0.001) in the untreated MI rats. In both icatibant and R-715 treated groups, the IL-10 mRNA expression was not significantly different from both sham-operated and untreated MI groups. The co-administration of both kinin receptor antagonists with MI vehicle treated and 40 mg·kg–1·day–1 omapatrilat-treated rats had no effect on the IL-10 mRNA expression, as compared with the untreated MI and the omapatrilat-treated groups, respectively.

**Discussion**

This study demonstrated that the vasopeptidase inhibitor omapatrilat and the ACE inhibitor captopril both improved early (4 days) post-MI survival when started 4 h post-MI. The use of BK antagonists had no significant effect on survival in untreated MI rats and MI omapatrilat-treated rats (not tested with captopril). This improvement in survival with omapatrilat and captopril is accompanied by a reduced LVEDP and pulmonary congestion. The use of BK antagonists had little effect on cardiac hemodynamics or morpho-
logic measurements. An acute MI significantly increases the expression of cardiac cytokines. Captopril significantly attenuates this activation, while omapatrilat has variable effects: sometimes increasing but generally not changing activation, depending on the cytokine measured and the dose of omapatrilat used. In nearly every case, the BK antagonists attenuated the increase in expression of cardiac cytokines. Taken together, these results would suggest that despite very marked differences in cardiac cytokine expression both captopril and omapatrilat improve early post-MI survival and cardiac function to a similar extent. It also demonstrates that under normal and MI circumstances, BK generation during this period does not affect survival, cardiac hemodynamics, or gross morphology, but has significant stimulatory effects on cardiac cytokine expression. Clearly the end result of the interaction between BK, cardiac cytokine expression, and the effects of these drugs in this setting are complex and linked with the convergence of numerous factors.

Recent studies have reported the temporal sequence of pro-inflammatory cytokine gene expression after permanent LAD occlusion (Herskowitz et al. 1995; Ono et al. 1998). In this study, we found a significant increase of TNF-α mRNA expression. The co-administration of icatibant and R-715 (BK antagonists) prevented the increase in TNF-α mRNA expression in untreated MI rats. Tiffany and Burch (1989) have reported that kinins stimulate TNF-α and IL-1 release from macrophages through B₁ kinin receptors. Moreover, infiltrating inflammatory cells such as macrophages, neutrophils, and fibroblasts are at their peak in the area of tissue injury 4 days post-MI (Sun et al. 1994). Similarly, several studies demonstrated that TNF-α is produced by cardiac myocytes (Giroir et al. 1992; Kapadia et al. 1995). The presence of functional B₂ receptors on cardiomyocytes have also been characterized (Minshall et al. 1995). Moreover, both B₁ and B₂ kinin receptors are up-regulated in both infarcted and non-infarcted areas (Tschöpe et al. 2000a, 2000b). It is thus possible that, after MI, endogenous kinins increase TNF-α mRNA expression and the release of the protein TNF-α from infiltrating inflammatory cells and cardio-

<table>
<thead>
<tr>
<th>Table 2. Morphologic parameters of heart at 4 days postinfarction.</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Sham</strong></td>
</tr>
<tr>
<td>Untreated ± BK antagonists</td>
</tr>
<tr>
<td>Omapatrilat</td>
</tr>
<tr>
<td>Captopril</td>
</tr>
<tr>
<td>Omapatrilat + BK antagonists</td>
</tr>
<tr>
<td><strong>MI</strong></td>
</tr>
<tr>
<td>Untreated ± BK antagonists</td>
</tr>
<tr>
<td>Omapatrilat</td>
</tr>
<tr>
<td>Captopril</td>
</tr>
<tr>
<td>Omapatrilat + BK antagonists</td>
</tr>
</tbody>
</table>

**Note:** Values are given as mean ± SE. LVW, left ventricular weight; BW, body weight; SW, scar weight; RVW, right ventricular weight; AW, atria weight; LW, lung weight.

*P < 0.05 vs. untreated ± BK antagonists.
**P < 0.01 vs. untreated ± BK antagonists
†P < 0.05 vs. respective Sham.
††P < 0.01 vs. respective Sham.

Fig. 2. RT-PCR analysis of TNF-α mRNA expression in the left ventricle 4 days after induction of myocardial infarction (MI) or after sham operation (SHAM). Drug treatments (10 mg·kg⁻¹·day⁻¹ omapatrilat (OMA-10), 40 mg·kg⁻¹·day⁻¹ omapatrilat (OMA-40), and 160 mg·kg⁻¹·day⁻¹ captopril (CAP-160)) were started 4 h after coronary artery ligation. B₁ and B₂ receptor antagonist (ANTA) treatments (2.5 mg·kg⁻¹·day⁻¹ each) were started during the surgery for MI. Values are means ± SE of 8 animals in each group. Values (TNF-α to GAPDH ratio, derived from RT-PCR) are arbitrary scanning units normalized to sham group 1. Significance is indicated by the following: a, P < 0.05 vs. SHAM group; b, P < 0.05 vs. MI group; c, P < 0.05 vs. MI+OMA-10 group; d, P < 0.05 vs. MI+OMA-40 group.
myocytes of the heart. Consistent with our data for a role of BK in the post-MI increase of TNF-α mRNA, we additionally found that BK receptor antagonists resulted in a significant reduction in cardiac TNF-α expression levels 4 days post-MI.

TGF-β1 is a major cytokine that regulates extracellular matrix protein formation in many cell types and tissues (Border and Noble 1994). TGF-β1 induces collagen mRNA expression and extracellular matrix formation in vitro in cultured rabbit or rat cardiac fibroblasts (Eghbali et al. 1991; Villarreal et al. 1996). Increased TGF-β1 mRNA expression has been demonstrated in the non-infarcted portion of the ventricle after MI (Thompson et al. 1988; Casscells et al. 1990; Hanatani et al. 1995). Our present data also indicate that an MI induced a significant increase of TGF-β1 mRNA expression in the LV non-infarcted rat tissue 4 days post-MI. However, as opposed to inflammatory cytokines, BK did not appear to play a role in this activation.

IL-10 inhibits the production of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 and up-regulates the expression of the IL-1 receptor antagonist (Howard and O’Garra 1992). Consequently, IL-10 could have beneficial effects in acute and chronic inflammatory diseases. In the present study, MI induced an increase of the IL-10 mRNA levels in LV non-infarcted tissue. This increased IL-10 expression could be a protective mechanism against uncontrolled activation of inflammatory cytokines. As with TGF-β1, the co-administration of both kinin receptor antagonists had no significant effect on IL-10 expression.

Our data show no significant effect of BK on survival rate or on any of the hemodynamic or morphologic parameters measured. This raises the question about the importance of BK on early (4 h to 4 days) post-MI prognosis. Because BK also significantly modified the expression of inflammatory cytokines, it also raises the question on the importance of suppressing the expression of these cytokines early post-MI. Indeed, although inflammatory cytokines are cardiodepressive and can contribute to adverse ventricular remodeling when chronically activated, it is possible that transient activation early post-MI may exert beneficial effects by stabilizing the necrotic scar and by stimulating post-MI healing (Mann 1996; Sack et al. 2000). Nevertheless, in this study we did not evaluate the effects of direct cytokine inhibition, and cannot rule out an important beneficial effect due to inhibition of inflammatory cytokines. Indeed, it is known that BK exerts a wide range of effects, some of which could mask potential beneficial effects of cytokine suppression.
Captopril has been shown to improve early post-MI survival in both the experimental and clinical setting (Pfeffer et al. 1985b, 1992). Indeed, in large clinical studies, most of the benefits of ACE inhibitors were found to occur within the first few days post-MI (GISSI-3 1994; ISIS-4 1995). The results of this study confirm the beneficial effects of captopril in this setting and indicate that they are accompanied by a reduction in LVEDP and pulmonary congestion. Thus, our data probably indicate that captopril may exert some of its beneficial effects by reducing the cardiac expression of various cytokines. Interestingly, captopril reduced cardiac cytokine expression despite increasing cardiac BK levels, a surprising result considering the suppression of cardiac cytokine expression with the BK receptor antagonists in our study. Moreover, a recent study showed that the TNF-α production by peripheral blood mononuclear cells in patients with CHF was markedly decreased by captopril (Zhao and Xie 2001), but other potent ACE inhibitors such as ramipril, perindopril, and lisinopril do not influence cytokine production (Schnidler et al. 1995). These findings suggest that other factors that suppress cytokine expression may be involved in captopril but not omapatrilat administration.

To our knowledge, this is the first report demonstrating the effects of omapatrilat on early experimental post-MI survival, cardiac hemodynamics, morphology, and cardiac cytokine expression. Omapatrilat administration resulted in an improvement in survival, cardiac hemodynamics, and morphology similar to that of captopril. This occurred despite marked differences in the way these drugs modified the expression of cardiac cytokines. Indeed, omapatrilat generally resulted in no change or, if anything, in an increase of the cardiac cytokine expression, while captopril reduced the expression of the same cytokines. That omapatrilat treatment leads to elevated expression of cardiac cytokines is not surprising considering the marked increase in cardiac BK that it causes (Blais et al. 2001). BK is a powerful proinflammatory substance, which resulted in increased expression of inflammatory cytokines in this study. Consistent with an important role of BK in the effects of omapatrilat on cardiac cytokine expression, the co-administration of BK receptor antagonists markedly attenuated the effects of omapatrilat on cardiac cytokine expression (TNF-α and TGF-β1).

The similar beneficial effects of omapatrilat and captopril on survival and cardiac hemodynamics despite their contrasting effects on cardiac cytokine expression suggests that the effects of cardiac cytokines in these settings are complex. Indeed, as already discussed, the effects of cardiac cytokines post-MI may be potentially beneficial or detrimental (Mann 1996; Sack et al. 2000). The release of cytokines from the myocardium following acute–subacute hemorrhage (Mann 1996; Sack et al. 2000). It seems that the cytokine–cardiac interaction early post-MI is quite complicated since the co-administration of BK receptor antagonists with omapatrilat resulted in an apparent attenuation of the beneficial effects of omapatrilat on survival. Clearly, the mechanisms for the beneficial effects of these drugs on early post-MI survival and their interaction with cardiac cytokine expression are complex and the result of the convergence of multiple competing mechanisms.

Acknowledgments

This study was supported by a Pharmaceutical Manufacturers Association of Canada – Medical Research Council of Canada grant to A. Adam and J.-L. Rouleau. C. Blais, Jr., and N. Lapointe are the recipients of a scholarship from the Fonds de la recherche en santé du Québec (FRSQ).

References


