THE EFFECTS OF PRE- PERI- AND POST-MYOCARDIAL INFARCTION TREATMENT WITH OMAPATRILAT IN RATS: EFFECTS ON SURVIVAL, ARRHYTHMIAS, FUNCTION AND REMODELLING


Running head: Vasopeptidase inhibition pre- peri- and post-myocardial infarction.

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Abstract

We have shown that the vasopeptidase inhibitor (VPI) omapatrilat improves peri-myocardial infarction (MI) survival, but the mechanisms involved and whether these effects are sustained remains to be determined, and are the subject of this study. Rats (n=279) received omapatrilat 20 mg/kg/day starting 7 days pre-MI, and continued peri- and post-MI, or no treatment (control). One group of rats had continuous ambulatory ECG and blood pressure recordings started 6 hours pre-MI and continued 24 hours post-MI when survival was evaluated, rats sacrificed and MI size evaluated. A second group had left ventricular (LV) remodelling evaluated by echocardiography at 30 days and, at 38 days had cardiac hemodynamics and morphology done, and survival evaluated. Survival 24 hours post-MI (N=267) improved with omapatrilat (60% versus 46% for control, p=0.0378). Over the next 37 days, there was no further improvement with omapatrilat, but the early benefit was sustained. Omapatrilat reduced MI size 24 hours post-MI, 36±2mm² versus 42±2mm² for controls, p=0.034. Omapatrilat reduced ventricular arrhythmia score 1 to 12 hours post-MI. Ompatrilat decreased blood pressure, but not during the first 24 hours post-MI. Omapatrilat reduced LV diastolic and systolic dimensions, and reduced LV and right ventricular (RV) weights as compared with control large MI, indicating a decrease in reactive hypertrophy. The improvement in cardiac remodelling was accompanied by improved cardiac hemodynamics. Thus, this study indicates that pre-, peri- and post-MI treatment with the VPI omapatrilat is beneficial on survival, ventricular arrhythmias, LV remodelling, and cardiac function.

Key Words: omapatrilat, myocardial infarction, remodelling, arrhythmias, vasopeptidase inhibitor.
During the acute phase of a myocardial infarction (MI), the major cause of death is ventricular arrhythmias. Indeed, nearly 70% of patients dying of an acute MI, die suddenly prior to arriving to hospital (11). The major goal of therapy once the patient reaches hospital is re-establishing cardiac perfusion in order to limit MI size. Once the MI is well established and the damage irreversible, one of the major objectives is to limit adverse LV remodelling in order to reduce the risk of heart failure and other post-MI complications.

Drugs such as angiotensin converting enzyme (ACE) inhibitors have been shown to be useful in improving outcome when started early post MI (1, 13, 18). A new class of drugs, the vasopeptidase inhibitors (VPI), simultaneously inhibit the activity of both the ACE and neutral endopeptidase (NEP) enzymes. In a previous study, we showed that, when started 4 hours post-MI, omapatrilat improved LV remodelling, LV function and survival (12). In another study, we demonstrated that the VPI omapatrilat reduced peri-MI mortality (4 days) in rats, and that this beneficial effect could be blocked by the simultaneous administration of B₁ and B₂ bradykinin (BK) receptor antagonists (3). However, the number of rats in the peri-MI study was relatively small, the mechanism by which BK was beneficial, and whether these early benefits were sustained or lost over time were not assessed.

This study was thus undertaken 1) to confirm whether omapatrilat improves peri- and early post-MI survival when given pre-, peri- and post-MI, and whether these early benefits are preserved over time; and, 2) to determine the mechanisms by which this occurs. The mechanisms that we evaluated in assessing why omapatrilat improved survival were based on knowledge of the fundamental mechanisms involved in early and late post-MI survival, that is, ventricular arrhythmias, MI size, LV remodelling and function. Our hypotheses were 1) that omapatrilat
would improve early post-MI survival, and that this benefit would be sustained: and, 2) that omapatrilat would reduce ventricular arrhythmias, reduce MI size, improve LV remodelling and function.
**MATERIALS AND METHODS**

**Animals and Drug-Regimen (figure 1):**

Spargue Dawley rats (Charles River, St-Constant, QC), 9 to 10 weeks old, had either the vasopeptidase inhibitor omapatrilat 20mg/kg/day (4, 5) or normal crushed laboratory chow started 7 days prior to MI surgery. Omapatrilat inhibits both ACE (IC$_{50}$ = 5 nM) and NEP (IC$_{50}$ = 9 nM), and was provided for research purposes by Bristol-Myers Squibb (Princeton, NJ). In order to assure adequate levels of omapatrilat peri-MI, an intraperitoneal injection of omapatrilat 2 mg/kg or saline (untreated control group) was administered 4 hours post-MI and again the next morning in survivors. Omapatrilat 20mg/kg/day or normal crushed laboratory chow was resumed post-MI and continued for 24 hours or 38 days according to the protocol (figure 1). 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 University Health Network (Toronto, ON).

**Experimental Myocardial infarction**

Rats were anesthetized with an intramuscular injection of a mixture of ketamine 50 mg/kg and xylazine 10 mg/kg and a MI was induced by ligating the left anterior descending coronary artery as described by Nguyen *et al* (17). Once awakened following surgery, the rats were injected intramuscularly with buprenorphine HCl 0.01 mg/kg (Reckitt Colman Pharmaceuticals, Richmond, VA) 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.

**24 Hour Peri-MI Arrhythmia, Blood Pressure, and Survival Protocol:**
Ambulatory ECG and Blood Pressure Monitoring

In order to assess the effects of the MI with and without omapatrilat on systemic arterial pressure and arrhythmias, 34 rats had surgery performed eight days pre-MI, prior to starting medications in order to install both continuous ECG and blood pressure monitoring (Data Sciences International, St-Paul, MN). Rats were anesthetized with an intramuscular injection of a mixture of ketamine 50 mg/kg (Rogar/STB, Montreal, Qc), and xylazine 10 mg/kg (Bayer Canada, Etobicoke, ON). For ECG and blood pressure monitoring, all MI>10 mm² (approximately 10% MI size by circumference) were considered for the analyses. Only one rat had a MI< 10 mm², and it was excluded from all analyses.

In order to obtain continuous electrocardiographic monitoring for arrhythmias, an hermetically sealed transmitter with a pair of helical wound flexible stainless steel wires (Data Sciences International, St-Paul, MN) was implanted subcutaneous for continuously ECG recording. The positive lead was placed in a V4-V5 position (the xiphoid space and caudal to the rib cage) and attached to the underlying tissue to prevent migration; the negative lead was secured in the area of the right shoulder. The bio-potential signal was digitized, amplified, and continuously emitted with the radio-frequency carrier.

In order to obtain continuous ambulatory blood pressure monitoring, an incision was made on the abdominal midline to permit access to the abdominal aorta. With the use of a bent needle as a catheter introducer, the blood pressure catheter was inserted upstream into the aorta. The body of the implant that contains the electronics for signal handling and radio-transmission was placed in the peritoneal cavity. The catheter insertion site was sealed and the abdomen closed. Once awakened following surgery, the rats were injected intramuscularly with buprenorphine HCl 0.01 mg/kg to reduce the pain during recovery. The rats were then housed in an individual cage placed on a receiver that continuously captures the radio-frequency signal from the transmitter.
and converts it into a serial bit stream (Dataquest A.R.T.2.2, DSI, St-Paul, Mn.). Ambient barometric pressure was also measured and subtracted from the telemetered pressure by data collection software to compensate for changes in atmospheric pressure.

Arrhythmia Analysis: The observer classified all arrhythmic events on ambulatory electrocardiographic recordings according to the guidelines provided by The Lamberth Conventions (24). Ventricular tachycardia (VT) was defined as 4 or more consecutive ventricular premature beats (premature QRS complexes in relation to the P wave). Ventricular fibrillation (VF) was defined as a signal that changed from beat to beat in rate and morphology or a signal in which individual QRS deflexions could not easily be distinguished from one another. The incidence and duration of arrhythmias was quantified as described by Curtis and Walker (7). As we did not measure individual premature beats, for this study, the scoring system started at 2, and this was given for 1 episode of spontaneously reverting VT or VF. A score of 3 was given for > 1 episode of VT or VF or both with a total combined duration of <60 seconds. A score of 4 was given for VT or VF or both with a combined total duration of 60-119 seconds, 5 for VT or VF or both of a combined duration of >119 seconds, 6 for fatal VF starting at > 15 min after occlusion, 7 for fatal VF starting at between 4 min and 14 min 59 s after occlusion, 8 for fatal VF starting at between 1 min and 3 min 59 s after occlusion and 9 for fatal VF starting < 1 min after occlusion. The average arrhythmia score was calculated for four post-MI time periods, 0 to 1 h, 1 to 6h, 6 to 12h, and 12 to24h. Rats that died during a time period were given a score of 9 for the following time periods.

Assessment of MI Size

Rats dying <4 hours post-MI were considered to have had a large MI. Rats dying between 4 and 24 hours or surviving 24 hours post-MI had their hearts removed for determination of infarct size
by triphenyl tetrazolium chloride (TTC). The LV was hand-cut into four slices before staining with TTC. Tissue slices were stained by incubation in 1% TTC for 10 to 15 minutes at 37°C and pH 7.8. The tissue was then bathed for 15 minutes in a 10% formalin phosphate buffer to enhance colour contrast. For each slice, the scarred area was photographed under a Leica M26 (Leica Microsystems Inc., Quebec, CA) and its surface was determined by planimetry (Labtronics Inc., Guelph, ON). Sections were traced on a calibrated digitizing tablet and morphologic variables calculated directly by computerized-planimetry with the Sigma Scan Pro software (Labtronics Inc., Guelph, Ontario, Canada).

38 days protocol (figure 1):

**Drug administration and experimental MI:**

One hundred fifty six rats were chosen for long-term (38 days) follow-up post-MI. These rats were randomly divided into the same two groups, omapatrilat (n=82) and control (n=74), 7 days prior to MI. Pre- peri- and post-MI drug administration, and the experimental MI were identical to that of the 24 hour protocol, except that rats had an echocardiogram at 30 days post MI and were treated for an additional of 37 days post-MI.

**Echocardiographic measurements:**

Thirty days post-MI, cardiac function and geometry were evaluated with an echocardiographic system equipped with a 15-MHz linear transducer (Acuson c256, Osiris Medical Inc., Toronto, ON), as described previously (23). All studies were performed with rats anesthetized with 2% halothane (Halocarbon Laboratories, Riveredge, NJ) using a vaporizer for halothane (Harvard Apparatus, Quebec, CA) and 0.2 l/min of oxygen. A comprehensive 2-dimensional study was performed for the measurement of the LV circumference and areas in both, systole and diastole.
LV systolic diameter (LVSD), LV diastolic diameter (LVDD) and wall thickness were measured in the short-axis M-mode using the right parassternal projection in a plane below the mitral valve and perpendicular to the LV. All primary measurements were traced manually and digitized by goal-directed, diagnostically driven software installed within the echocardiographic system. An average of 3 beats were used for the short-axis M-mode measurements.

**Cardiac hemodynamic measurements**

Thirty-eight days post-MI, the rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg), and xylazine (10 mg/kg) mixture. The LV and right ventricular (RV) pressures were measured by a Millar Micro-Tip Catheter Transducer (Millar Instruments, Houston, TX) with a pressure sensor at the tip. The catheter was inserted into the right jugular vein and carotid artery and advanced to the right ventricle (RV) and LV, respectively. Systolic and diastolic arterial pressures were measured in the carotid artery before being advanced to the LV. Ventricular pressures and the maximum rate of pressure rise (+dP/dt) and decline (-dP/dt) were both measured. The pressures were recorded on a Gould 2600S recorder (Gould, Cleveland, OH).

**Morphologic Measurements:**

In rats surviving to 38 days, the heart was removed, rapidly rinsed in saline solution, and dissected into atria, RV, LV, septum and scar. All portions of the heart, as well as the lungs, were then weighed individually. Animals were then classified according to MI size according to scar surface as described above. The rats dying later than 24 hours post-MI but prior to hemodynamic monitoring had morphologic assessment for classification of MI size by assessing % of LV circumference infarcted, as previously described (2), but were not used for other measurements except for survival. Rats surviving 38 days had a large MI defined as a left
ventricular scar surface of $\geq 35 \text{ mm}^2$, rats with a moderate MI as $<35 \text{ mm}^2$. Surface area of a scar and MI size by % of LV circumference infarcted are approximately the same in our experience (17).

**Statistical analysis**

All data are expressed as means ± SEM. Statistical significance was calculated using a Student’s unpaired $t$-test. 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 (figure 2)

24 hours post MI survival (N=279): The 24 sham-operated rats all survived. The overall survival 24 hours post- (moderate and large) MI in the control group (N= 73 deaths, 61 survivors) was 46%, which was less than omapatrilat (N= 49 deaths, 72 survivors) where 60% survived, p=0.0378 versus control.

24 hours to 38 days post-MI survival (N=106): Rats in the sham groups all survived until the end of the study. In the control MI rats, 4 of 47 died, 91% survival, similar to omapatrilat, where 2 of 47 rats with an MI died, 96% survival, p=0.3914 versus control.

24 Hour Arrhythmia and Ambulatory Blood Pressure:

Incidence and Duration of Arrhythmias: (figure 3)

No VT or VF was recorded during the 6 hour baseline recording prior to coronary artery occlusion. During the first hour post-MI, control rats had an average arrhythmia score of 4±0.3 as compared with 3±0.4 for omapatrilat, p=0.166. From 1 to 6 hours and 6 to 12 hours post-MI, control rats had a greater arrhythmia score than omapatrilat, 5±0.9 versus 2±0.8, p=0.008 and 7±0.6 versus 4±0.9, p=0.028 respectively. There was no difference in arrhythmia score 12 to 24 hours post-MI, 6±1.1 for control versus 5±1.0 for omapatrilat, p=0.826.

Ambulatory Blood Pressure (figure 4):

Treatment with omapatrilat 20 mg/kg/day resulted in a significant decrease in systolic and diastolic arterial pressures prior to MI. This difference persisted throughout the experimental period in the sham-operated rats. During the post-MI period of monitoring, 0 to 24 hours, blood
pressure was significantly reduced in the control group. Treatment with omapatrilat did not result in a further decrease in blood pressure 0 to 24 hours post MI.

**MI Size**

If one considers only rats in the 24 hour protocol that survived the full 24 hours, there was no difference in MI size between the control and omapatrilat, $21\pm2$ mm$^2$ versus $20\pm1$ mm$^2$ respectively. If one then considers both survivors and rats dying between 4 and 24 hours, rats receiving omapatrilat had smaller MI, $36\pm2$ mm$^2$ versus $42\pm2$ mm$^2$ for control MI, $p=0.034$.

**38 Day Protocol:**

**Echocardiographic Measurements (table 1)**

In sham rats, the only difference between control and omapatrilat was a decrease in posterior wall thickness in the omapatrilat group.

As compared with sham, control moderate MI rats had dilatation of the LV, with endocardial circumference in systole and diastole, and LVDD and LVSD all increasing. Posterior wall thickness did not change, but anterior wall thickness (area of the MI) decreased. Omapatrilat treated rats with a moderate MI had similar changes, except that the increase in LVDD was not significant as compared with their omapatrilat sham counterparts, and posterior wall thickness decreased as compared with moderate MI controls.

As compared with control sham and moderate MI groups, control large MI rats had further LV dilatation as reflected by an increase in endocardial circumference in systole and diastole, and LVDD and LVSD. Anterior wall thickness resembled that of moderate MI, but posterior wall thickness decreased as compared with sham and moderate MI. Omapatrilat attenuated LV
dilatation, and reduced posterior wall thickness but not the anterior wall thickness as compared with control large MI.

**Hemodynamic Measurements (table 2)**

In sham rats, omapatrilat resulted in a decrease in both systolic and diastolic arterial pressure. LV systolic pressure (LVSP) and the LV +dP/dt were also decreased. No other changes in the measured hemodynamic parameters were observed.

As compared with control sham, control moderate MI rats had no change in the measured hemodynamic parameters, with the exception of LV +dP/dt that was decreased and right ventricular end-diastolic pressure (RVEDP) that was increased. As compared with control moderate MI rats, omapatrilat resulted in a significant decrease in systolic and diastolic pressure, as well as a decrease in LVSP and RVEDP. Rats with moderate MIs treated with omapatrilat had no hemodynamic differences as compared with their sham counterparts.

As compared with control sham, control large MI rats had an increased in LV end diastolic pressure (LVEDP) and in RV systolic pressure (RVSP), and a decrease in LVSP and in LV +dP/dt, all compatible with cardiac dysfunction. Omapatrilat treatment resulted in a further decrease in systolic and diastolic pressure, as well as in LVSP, LV +dP/dt and RVSP. Omapatrilat treatment prevented all increase in RV pressures as compared with sham or moderate MI.

**Morphological Studies (table 3)**

In sham rats, omapatrilat resulted in a decrease in total LV weight (septum + LV) to body weight ratio (TLVW/BW) as compared with the control group. No other change in the measured morphologic parameters was observed in this category of rats.
Control moderate MI rats were not significantly different from their sham counterparts, except for the presence of a LV scar. The other morphologic differences between control and omapatrilat moderate MI were similar to those found in their sham counterparts except the body weight that was decreased.

Control large MI rats had an increase in scar surface and weight, in atrial weight/BW (AW/BW), and in RV weight/BW (RVW/BW) as compared with their sham and moderate MI counterparts. Also, compatible with lung congestion, they had an increase in wet lung weight/BW (lung W/BW) as compared with their sham counterparts. As compared with control large MI, omapatrilat decreased body weight, and TLVW/BW (scar + septum + LV) similar to sham and moderate MI. MI size, and scar characteristics were similar, but RVW/BW, AW/BW and Lung W/BW were all decreased as compared with control large MI suggesting a decrease in hypertrophy and pulmonary congestion.

If one considers only rats that survived 38 days with an MI, then there was a statistically borderline decrease in MI size with omapatrilat (40±2 mm² versus 50±3 mm², p=0.0523). If one then considers both survivors and rats dying between 24 hours and 38 days, the trend in favour of smaller MI with omapatrilat becomes significant (40±2 mm², versus 49±2 mm², p=0.0272).
**DISCUSSION**

In this study, omapatrilat pre-, peri- and post-MI resulted in an improvement in survival. This improvement in survival was accompanied by a decrease in severity of ventricular arrhythmias, a decrease in MI size and an improvement in LV remodelling and cardiac function. Interestingly, omapatrilat did not further reduce systemic arterial pressure during the first 24 hours post-MI. Taken together, these data would suggest that the effects of omapatrilat are beneficial in the acute and chronic post-MI periods.

Omapatrilat given pre-, peri-, and post-MI improved survival. This improvement in survival was largely the result of better survival during the first 24 hours of the MI, but was sustained over the 38 days of the study. In a previous study of a relatively small number of rats, we found a similar beneficial effect of omapatrilat on four day post-MI survival, and found this beneficial effect to be eliminated by the simultaneous administration of B1 and B2 BK receptors antagonists (3) suggesting that BK is involved in the cardioprotective effects of omapatrilat in this setting. In the present study, we confirm these beneficial effects of omapatrilat in a larger number of rats, found that these early beneficial effects are sustained for a longer period post-MI, and identified three mechanisms, which were likely inter-related, by which omapatrilat may have exerted these beneficial effects. These include a reduction in MI size, a reduction in ventricular arrhythmias, and an improvement of ventricular remodelling.

Omapatrilat reduced MI size which clearly contributed to improved survival. Whether one considers all rats, survivors and non-survivors, 24 hours post-MI, or rats that survived 38 days or died between 24 and 38 days post-MI, we found a reduction in MI size. Taken together, these findings would suggest that the early and sustained benefits of omapatrilat on survival were
largely the result of a reduction in MI size. Although the reduction in MI size with omapatrilat was likely the convergence of a number of factors, our previous study indicating that blocking the effects of BK eliminated the benefits of omapatrilat on survival would suggest that BK may be involved. Intracoronary injection of BK in acute rabbit studies reduces infarct size to the same extent as the ACE inhibitor ramaprilat, whereas the BK B_2 receptor antagonist HOE-140 abolished the effect of ramaprilat (9). Compatible with this are experimental studies where arrhythmias and MI size are increased when BK is inhibited (14), studies of BK knockout mice demonstrating increased peri-MI mortality (25) and a clinical study where an increase in plasma kallikrein levels was positively correlated with early survival post-MI (10). Multiple mechanisms may be involved in BK-mediated protection against ischemic damage and include improved coronary and capillary nutritional flow (15, 16), and kinin-induced changes in cardiac metabolism, such as the preservation of high energy-enriched phosphates and increased myocardial glucose uptake and use (15, 19).

Other potential mechanisms by which omapatrilat may have reduced MI size, include hemodynamic and neurohumoral effects. To our surprise, during the first 24 hours post-MI, omapatrilat did not further reduce systemic arterial pressure. One possible explanation for this finding is that the dose of omapatrilat was inadequate. Against this, is the reduction of systemic arterial pressure with omapatrilat prior to the MI and during studies done 38 days post-MI. Nevertheless, during the first 24 hours post-MI, rats do not eat normally and may not have consumed enough omapatrilat to reach therapeutic levels. In order to counter this problem we gave rats omapatrilat 2mg/kg intra-peritoneal 4 hours post-MI and again the next morning. Although this was sufficient to reduce pressures in sham operated rats, it may have been inadequate in rats with an MI. In any case, it would appear that, at least in the present study, the reduction in MI size with omapatrilat was not the result of a decrease in arterial pressure.
Reduction in MI size could also have resulted from the direct neurohumoral effects of omapatrilat. These include a reduction in angiotensin II and an increase in natriuretic peptides (8, 20).

Omapatrilat reduced the severity of ventricular arrhythmias. This reduction in arrhythmias was largely confined to the period of 1 to 12 hours post-MI. At least part of this reduction in arrhythmias with omapatrilat resulted from its beneficial effect on MI size, but, it may also have partially been the result of an anti-arrhythmic effect of BK. Because BK may be involved in reducing MI size, it is difficult to determine what proportion of its anti-arrhythmic effect is the result of a reduction in MI size and how much is the result of a direct anti-arrhythmic effect. Nevertheless, there is a significant body of evidence suggesting that BK reduces ventricular arrhythmias peri-MI. Low doses of BK have been shown to reduce ischemic arrhythmias and to improve myocardial electrical stability while not having an effect on coronary blood flow (15, 21, 22). BK also reduces the severity of ventricular arrhythmias induced by short-term occlusion/reperfusion, even before necrosis occurs (15, 22), and blockade of BK increases ventricular arrhythmias (14). Finally, BK appears to largely mediate the ACE inhibitor-induced reduction in norepinephrine and ventricular arrhythmias that occur during ventricular ischemia, as their protective effects are inhibited by blocking BK (6).

In this study, we also found that omapatrilat attenuated LV dilatation and helped preserve cardiac function 38 days post-MI, a third mechanism that could have resulted in improved survival. The control large MI group had marked LV dilatation which was accompanied by a reduction in posterior wall thickness, suggesting some slippage of the cardiomyocytes in that area. Omapatrilat attenuated LV dilatation and hypertrophy in all groups. Because we did not assess LV remodelling 24 hours post-MI, we cannot be certain to what extent this contributed to the early benefits of omapatrilat. This improvement in ventricular remodelling was not accompanied
by an improvement in LV function, but was accompanied by a decrease in RVSP and a decrease in pulmonary wet weight suggesting a decrease in pulmonary congestion. This beneficial effect of omapatrilat did not result in improved survival from 24 hours to 38 days post-MI. The major reason for this is the small number of deaths in the control group (4 of 47) during this period, making our study woefully underpowered to determine a difference in survival. It is possible that with a longer follow-up, the beneficial effects of omapatrilat on LV remodelling would have resulted in improved survival. In support of this is the late survival benefit with the ACE inhibitor captopril in the survival and ventricular enlargement study (SAVE) (18).

**Conclusions**

This study indicates that pre-, peri- and post-MI treatment with the VPI omapatrilat improves survival. Along with improved survival, omapatrilat reduces MI size, decrease ventricular arrhythmias and results in improved LV remodelling and cardiac function. The results of this study, combined with our pervious work would suggest that VPI omapatrilat is beneficial when given pre-, peri- and/or post-MI in rats.
Acknowledgements

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FIGURE TO LEGENDS

**FIGURE 1:** Flow diagram of the various groups of rats according to the presence of myocardial infarction (MI) and treatment group. Oma=omapatrilat, H=hours, D=days.

**FIGURE 2:** Comparison between survival of rats with a large MI control and treated with omapatrilat 20 mg/kg/day at 4 and 24 hours post MI.

**FIGURE 3:** Average arrhythmia score of rats with moderate and large myocardial infarction 24 hour post myocardial infarction (MI).

**FIGURE 4:** Systolic (top) and diastolic (bottom) systemic blood pressure in rats with moderate and large myocardial infarction (MI) during the first 24 hour post myocardial infarction.
Table 1. Echocardiographic Measurements at 38 Days Postinfarction

<table>
<thead>
<tr>
<th></th>
<th>LVDD (cm)</th>
<th>LVSD (cm)</th>
<th>Anterior Wall Thickness (mm)</th>
<th>Posterior Wall Thickness (mm)</th>
<th>Cir Diastole Endocardium (cm)</th>
<th>Cir Systole Endocardium (cm)</th>
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<td>Control (N=6)</td>
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<td><strong>Moderate MI</strong></td>
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<td>0.89±0.03 †</td>
<td>1.02±0.01 †</td>
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<td>3.38±0.07 *†#</td>
<td>3.06±0.07 *†#</td>
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LVDD indicates left ventricular diastolic dimension; LVSD, left ventricular systolic dimension in short axis, just below plane of mitral valve; Cir, circumference in 2 dimensions.

Values are given as mean ± SEM. *P < 0.05, vs control; †P < 0.05, vs sham; #P < 0.05, vs moderate MI.
Table 2. Hemodynamic Measurements at 38 Days Postinfarction

<table>
<thead>
<tr>
<th></th>
<th>HR, bpm</th>
<th>SAP, mmHg</th>
<th>SAP, mmHg</th>
<th>LVSP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>LV+DP/dt, mmHg</th>
<th>RVSP, mmHg</th>
<th>RVEDP, mmHg</th>
<th>RV+DP/dt, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham</strong></td>
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</tr>
<tr>
<td>Control (N=6)</td>
<td>302±10</td>
<td>116±6.2</td>
<td>86±4.2</td>
<td>105±6</td>
<td>8±1</td>
<td>7552±510</td>
<td>27±1</td>
<td>5±1</td>
<td>1791±51</td>
</tr>
<tr>
<td>Omapatrilat (N=6)</td>
<td>311±13</td>
<td>88±2.2*</td>
<td>65±3.6*</td>
<td>85±5*</td>
<td>9±1</td>
<td>5705±402*</td>
<td>26±1</td>
<td>5±1</td>
<td>1715±57</td>
</tr>
<tr>
<td><strong>Moderate MI</strong></td>
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<tr>
<td>Control (N=13)</td>
<td>314±11</td>
<td>117±4.4</td>
<td>91±3.6</td>
<td>100±4</td>
<td>10±1</td>
<td>5889±176†</td>
<td>28±1</td>
<td>9±1†</td>
<td>1713±70</td>
</tr>
<tr>
<td>Omapatrilat (N=19)</td>
<td>315±12</td>
<td>96±3.8*</td>
<td>72±3.0*</td>
<td>82±3*</td>
<td>9±1</td>
<td>5246±236</td>
<td>25±1</td>
<td>6±1*</td>
<td>1787±54</td>
</tr>
<tr>
<td><strong>Large MI</strong></td>
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<tr>
<td>Control (N=30)</td>
<td>357±14</td>
<td>109±2.7†#</td>
<td>83±2.1</td>
<td>86±2†#</td>
<td>13±1†#</td>
<td>4835±135†#</td>
<td>32±1†</td>
<td>9±1</td>
<td>1952±61</td>
</tr>
<tr>
<td>Omapatrilat (N=26)</td>
<td>368±17</td>
<td>92±3.4*</td>
<td>66±2.6*</td>
<td>75±2*†#</td>
<td>12±1†#</td>
<td>4280±129*†#</td>
<td>27±1†</td>
<td>7±1</td>
<td>1841±64</td>
</tr>
</tbody>
</table>

HR indicates heart rate; SAP, systolic arterial systemic pressure; DAP, diastolic arterial systemic pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LV +dP/dt, maximum rate of left ventricular pressure development; RVSP, right ventricular systolic pressures; RVEDP, right ventricular end-diastolic pressure; RV+dP/dt, maximum rate of right ventricular pressure development.

Values are given as mean ± SEM. *P < 0.05, vs control; † P < 0.05, vs sham; # P < 0.05, vs moderate MI.
Table 3. Morphology Parameters at Time of Induced death

<table>
<thead>
<tr>
<th></th>
<th>Infarct Surface (mm²)</th>
<th>Body W (g)</th>
<th>Scar W (mg)</th>
<th>Total LVW/BW, mg/g</th>
<th>RVW/BW, mg/g</th>
<th>AW/BW, mg/g</th>
<th>Lung W/BW, mg/g</th>
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<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td><strong>Sham</strong></td>
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<td></td>
</tr>
<tr>
<td>Control (N=6)</td>
<td>-</td>
<td>496±11</td>
<td>-</td>
<td>2.06±0.11</td>
<td>0.49±0.03</td>
<td>0.14±0.01</td>
<td>2.95±0.19</td>
</tr>
<tr>
<td>Omapatrilat (N=6)</td>
<td>-</td>
<td>444±12</td>
<td>-</td>
<td>1.60±0.06*</td>
<td>0.39±0.03</td>
<td>0.12±0.01</td>
<td>3.29±0.13</td>
</tr>
<tr>
<td><strong>Moderate MI</strong></td>
<td></td>
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</tr>
<tr>
<td>Control (N=13)</td>
<td>28.33±2</td>
<td>473±12</td>
<td>0.07±0.01</td>
<td>1.94±0.06</td>
<td>0.49±0.01</td>
<td>0.16±0.01</td>
<td>2.96±0.09</td>
</tr>
<tr>
<td>Omapatrilat (N=19)</td>
<td>27.30±1</td>
<td>432±9*</td>
<td>0.07±0.01</td>
<td>1.64±0.03*</td>
<td>0.47±0.02</td>
<td>0.14±0.01</td>
<td>3.16±0.06</td>
</tr>
<tr>
<td><strong>Large MI</strong></td>
<td></td>
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</tr>
<tr>
<td>Control (N=30)</td>
<td>54.84±3#</td>
<td>477±7</td>
<td>0.11±0.01#</td>
<td>1.92±0.04</td>
<td>0.67±0.05†#</td>
<td>0.24±0.02†#</td>
<td>4.41±0.33†</td>
</tr>
<tr>
<td>Omapatrilat (N=26)</td>
<td>48.88±2#</td>
<td>438±5*</td>
<td>0.10±0.01#</td>
<td>1.56±0.04*</td>
<td>0.54±0.02*†#</td>
<td>0.17±0.01*</td>
<td>3.61±0.20*</td>
</tr>
</tbody>
</table>

W indicate weight; Total LVW, Total left ventricular weight (scar, septum and LV); RVW, right ventricular weight; AW, atria weight.

Values are given as mean ± SEM. *P < 0.05, vs control; † P < 0.05, vs sham; # P < 0.05, vs moderate MI.
Pre-Treatment with Oma or Untreated

MI
N=272

24 hours Protocol
N=116

Dead
Oma N=28
Untreated N=44

Large MI
Oma N=22
Untreated N=12

Moderate MI
Oma N=3
Untreated N=2

Sham
Oma N=1
Untreated N=4

38 days Protocol
N=156

Dead 0 to 38D
Oma N=23
Untreated N=33

Large MI
Oma N=26
Untreated N=30

Moderate MI
Oma N=19
Untreated N=13

Sham
Oma N=6
Untreated N=6

Dead 24H to 38D
Oma N=2
Untreated N=4
Survival of Rats 4 and 24 Hour Post MI

* p<0.05 vs Control
Figure 3

Average Arrhythmia Score 24 hours Post MI

* p<0.05 vs Control
Systemic Blood Pressure 24h Post MI

* p<0.05 vs Control