Effects of pre-, peri-, and postmyocardial infarction treatment with losartan in rats: effect of dose on survival, ventricular arrhythmias, function, and remodeling

Ali Pourdjabbar,1 Thomas G. Parker,1 Quang Trinh Nguyen,2 Jean-Francois Desjardins,1 Nathalie Lapointe,2 James N. Tsoporis,1 and Jean-Lucien Rouleau3

1Division of Cardiology and Department of Medicine, St. Michael's Hospital, University of Toronto, Toronto, Ontario; 2Division of Cardiology, University Health Network, Toronto, Ontario; and 3Department of Medicine, Montreal Heart Institute, Montreal, Quebec, Canada

Submitted 6 July 2004; accepted in final form 10 November 2004

Pourdjabbar, Ali, Thomas G. Parker, Quang Trinh Nguyen, Jean-François Desjardins, Nathalie Lapointe, James N. Tsoporis, and Jean-Lucien Rouleau. Effects of pre-, peri-, and postmyocardial infarction treatment with losartan in rats: effect of dose on survival, ventricular arrhythmias, function, and remodeling. Am J Physiol Heart Circ Physiol 288: H1997–H2005, 2005. First published November 11, 2004; doi:10.1152/ajpheart.00671.2004.—Angiotensin receptor blockers (ARBs) reduce adverse left ventricular (LV) remodeling and improve LV function and survival when started postmyocardial infarction (MI). ARBs also reduce ventricular arrhythmias during ischemia-reperfusion injury when started pre-MI. No information exists regarding their efficacy and safety when started peri-MI and continued peri- and post-MI. We evaluated whether the ARB losartan improves the outcome when started pre-MI and continued peri- and post-MI. Male Wistar rats (n = 502) were treated for 7 days pre-MI with losartan at a high dose (30 mg·kg⁻¹·day⁻¹), progressively increasing dose (3 mg·kg⁻¹·day⁻¹ increased to 10 mg·kg⁻¹·day⁻¹ 10 days and 30 mg·kg⁻¹·day⁻¹ 20 days post-MI), or no treatment. Ambulatory systolic blood pressure and Holter monitoring were performed for 24 h post-MI. Echocardiography was done 30 days post-MI, and LV remodeling, cardiac hemodynamics, and fetal gene expression were assessed 38 days post-MI. High-dose losartan reduced 24-h post-MI survival compared with the progressive dose and control (21.9% vs. 36.6% and 38.1%, P = 0.033 and P = 0.009, respectively). This was associated with greater hypotension in the high dose and no change in ventricular arrhythmias in all groups. In 24-h post-MI survivors, the progressive dose group had reduced mortality from 24 h to 38 days (8.5% vs. 28.6% for control vs. 38.9% for high dose, P = 0.032 and P = 0.01, respectively). Survivors of both losartan groups demonstrated improved LV remodeling, cardiac hemodynamics, preserved GLUT-4, and reduced cardiac fetal gene expression. Pretreatment with ARBs does not reduce 24-h post-MI ventricular arrhythmias or survival, and high doses increase mortality by causing excessive hypotension. In 24-h post-MI survivors, progressively increasing doses of losartan have multiple beneficial effects, including improved survival.

Angiotensin II receptor blockers; heart failure; infarction; remodeling; hypotension

During the acute phase of a myocardial infarction (MI), ventricular arrhythmias are the principle cause of death. Indeed, nearly 70% of patients dying of an acute MI die suddenly before arriving to the hospital (10). Safe and effective reduction in peri-MI arrhythmias is thus an essential strategy in improving peri-MI survival. Once a patient reaches the hospital, the major goal of therapy is reestablishing cardiac perfusion to limit MI size. Once the MI is well established and the damage irreversible, it is important to limit adverse left ventricular (LV) remodeling to reduce the risk of heart failure. Indeed, the extent of LV dilation is the most powerful predictor of long-term prognosis post-MI (22).

The acute phase of the MI is characterized by neurohumoral activation, a process that lasts for days and then generally subsides over days to weeks, unless heart failure develops, in which case there is chronic progressive neurohumoral activation. The renin-angiotensin system, particularly ANG II, is upregulated peri- and post-MI. Angiotensin receptor blocker blockers (ARBs) have been shown to improve the outcome when started early post-MI (21). One of the proposed mechanisms for these beneficial effects is improved LV remodeling (24), but other mechanisms are also possible (15) but not well worked out. It is uncertain as to what effect the presence (pretreatment) of an ARB would have in the setting of an acute MI. One possibility is that it would exert a beneficial effect. In favor of this is a report of the ARB losartan decreasing peri-MI arrhythmias during cardiac ischemia-reperfusion (28). However, it is also possible that they induce adverse effects due to excessive hypotension, as has been shown to occur with the angiotensin-converting enzyme inhibitor (ACEi) enalapril (26).

In the two studies that compared ARBs with ACEi therapy, drugs were initiated in small doses to avoid the adverse effects of excessive hypotension observed in the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II) trial (26). The dose was then progressively increased. In the Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan (OPTIMAAL), the ARB did not perform as well as the ACEi (7). One proposed hypothesis is that the dose of the ARB was inadequate (6). Consistent with this hypothesis, a larger dose of an ARB was found to be equivalent to an ACEi in the valsartan in acute myocardial infarction (VALIANT) trial (21). Thus avoiding excessively high doses of an ARB early post-MI and assuring the use of higher doses later post-MI may be important to obtain the optimal effects of ARBs post-MI.

Thus, in the present study, we chose to mimic the proposed optimal clinical use of ARBs post-MI that is starting with...
smaller known effective doses and progressively increasing to a high dose of the ARB losartan. We compared these effects with early and sustained high doses of losartan and with no treatment at all. We compared early (24 h) post-MI ventricular arrhythmias, systemic blood pressure and survival, and late post-MI LV remodeling, function, MI size, and fetal gene expression. We hypothesized that progressive doses of losartan would exert beneficial effects that would be superior to both control and early large doses of losartan.

MATERIALS AND METHODS

Animals and Drug Regimen

Male Wistar rats (Charles River, St. Constant, Quebec, Canada) weighing 160–200 g were assigned to one of three groups: losartan (Merck Frosst, Montreal, Quebec, Canada) at 30 mg·kg⁻¹·day⁻¹ (high dose) or 3 mg·kg⁻¹·day⁻¹ with an increase to 10 mg·kg⁻¹·day⁻¹ on day 10 post-MI and to 30 mg·kg⁻¹·day⁻¹ on day 20 post-MI (progressive dose) or control. The low and high doses of losartan were chosen based on a previous report demonstrating cardioprotective effects in rats after an MI (4, 24). The increasing dose of losartan over time post-MI was chosen to simulate the clinical situation where losartan is started low and increased after a large MI. Losartan was administered in the drinking water starting 7 days before the MI surgery. To assure adequate levels of losartan peri-MI and early post-MI, losartan (high dose: 15 mg/kg, 0.25 ml; low dose: 1.5 mg/kg, 0.25 ml) or vehicle (0.9% saline) was administered by oral gavage in the evening after the MI and again twice the next day, after which the medication was once again administered in the drinking water and continued for the duration of the specific protocol. All of the animal experiments followed the guidelines of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the University Health Network (Toronto, Ontario, Canada).

Experimental Myocardial Infarction

Rats were anesthetized with an intramuscular injection of a mixture of 50 mg/kg ketamine (Rogar/STB, Montreal, Quebec, Canada) and 10 mg/kg xylazine (Bayer Canada, Etobicoke, Ontario, Canada). MI was induced by ligation of the left anterior descending coronary artery as described previously by Nguyen et al. (19). Sham-operated rats underwent a similar procedure except the silk suture was not tied. Once awakened following the surgery, the rats were injected with 0.01 mg/kg buprenorphine HCl.

24-Hour Protocol

Ambulatory ECG and blood pressure monitoring. Ten days before the MI, with the rats (n = 48) under ketamine and xylazine anesthesia, a transmitter was implanted in the peritoneal cavity and anchored to the abdominal musculature. The implants had a pair of flexible stainless steel wires and leads (Data Sciences International, St. Paul, MN) implanted subcutaneously for continuous ECG monitoring, and a blood pressure catheter was inserted into the descending aorta, as previously described (14). The body of the implant that contains the electronics for signal handling and radiotransmission was placed in the peritoneal cavity. Rats were then housed in an individual cage placed on a receiver that continuously captures the radiofrequency signal from the transmitter and converts it into a serial bit stream (Dataquest A.R.T.2.2, Data Sciences International). 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. Monitoring of arrhythmias was started 6-h pre-MI until the time of death or 24-h post-MI. The observer classified all arrhythmic events on ambulatory electrocardiographic recordings according to the guidelines provided by The Lambeth Conventions and Curtis and Walker (3) with minor modifications as previously described by Lapointe et al. (14). The average arrhythmia score was calculated 6 h before MI and for 4 post-MI time periods 0–1, 1–6, 6–12, and 12–24 h.

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

38-Day Protocol

Male Wistar rats (n = 454) were randomly assigned into three groups: high-dose losartan (30 mg·kg⁻¹·day⁻¹) (n = 103), low-dose losartan (3 mg·kg⁻¹·day⁻¹ increasing gradually to 30 mg·kg⁻¹·day⁻¹) (n = 161), or control (n = 190) administered as described above (Fig. 1).

To verify if the chosen low dose of losartan blocked the ANG II signaling pathway, a dose-response curve to 6.5–650 ng/kg ANG II (Sigma, lot no. 31K51142) was constructed in 16 rats treated with 3 mg·kg⁻¹·day⁻¹ of losartan. The pressor response to ANG II was significantly attenuated for all doses of ANG II compared with control (results not shown).

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, Toronto, Ontario, Canada). All studies were performed with rats anesthetized with 2% halothane (Halocarbon Laboratories, Riveredge, NJ) using a vaporizer for halothane (Harvard Apparatus, Quebec, Canada) and 0.2 l/min of oxygen. A comprehensive two-dimensional study was performed for the measurement of the LV circumference and areas in both systole and diastole. LV systolic diameter (LVSD) and LV diastolic diameter (LVDD) were measured in the short-axis M-mode as previously described (14).

Cardiac hemodynamic measurements. Thirty-eight days post-MI, 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 microtip catheter transducer, which was inserted into the right jugular vein and carotid artery and advanced to the RV and LV, respectively. Systolic and diastolic arterial pressures were measured in the aorta before being advanced to the LV. The maximum rate of pressure rise (+dP/dt) and decline (−dP/dt) were also measured. The pressures were recorded on a Gould 2600S recorder (Cleveland, OH). Once the hemodynamic measurements were completed, rats were euthanized and used for either assessment of LV remodeling by passive pressure-volume relationship or morphological assessment of cardiac hypertrophy.

Morphological assessment of cardiac hypertrophy. 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 and frozen at −80°C. MI size was assessed by measuring the surface area of the scar. Before being weighed, the scar was pinned on paper and its surface area measured by planimetry (Labtronics). A large MI was defined as an infarct size ≥0.8 cm², a moderate MI was defined as an infarct size <0.8 cm², and a sham was defined as having no scar. In our experience (19), a scar area of ≥0.8 cm² is equivalent to an MI of ≥35% of LV circumference. Rats...
dying later than 24 h post-MI but before hemodynamic monitoring had morphological assessment for classification of MI size by assessing the percentage of LV circumference infracted, as previously described (3) but were not used for other measurements except for survival.

Passive pressure-volume relationship and ventricular remodeling. Because the number of rats surviving the 38-day protocol in the high-dose ARB group was low, only control and low-dose losartan groups were used for this protocol. Hearts were removed after cardiac hemodynamic measurements, rinsed in a saline solution, and then arrested in a supersaturated solution of KCl, after which passive pressure volume was assessed as previously described (2). After the assessment of the pressure-volume relationship, the hearts were filled with saline to a pressure of 15 mmHg and sealed and fixed in 10% formalin phosphate solution for 24 h. The heart was then cut halfway between the base and apex, and two slices were obtained 1-mm above and below the middle cut and stained with hematoxylin-phloxine saffron (HPS) for assessment of MI size as previously described (2). With the use of this method, a large MI was defined as that having a LV scar of \( \frac{35}{100} \) of the ventricular cross-sectional circumference, a moderate MI as having an LV scar \( \frac{35}{100} \) to \( \frac{70}{100} \), and sham having no scar.

RNA isolation and purification. Total RNA was isolated from treated and untreated tissues from the septum of hearts, using the TRIzol method. RNA samples were further subjected to RNase-free DNase I treatment at 37°C for 30 min and then purified with phenol chloroform and isoamyl alcohol, precipitated, and dissolved in nuclease-free water. RNA was quantified with GeneQuant spectrophotometer (Amersham Pharmacia Biotech, Piscataway, NJ), and RNA integrity was confirmed by running it on a formaldehyde gel. The isolated total RNA was used in real-time quantitative RT-PCR experiments.

Real-time quantitative RT-PCR. Thermoscript one-step quantitative RT-PCR with platinum Taq kit was used to synthesize cDNA and subsequent real-time quantitative RT-PCR (Applied Biosystems, Foster City, CA). The gene-specific sequences of oligonucleotide primers (100 nM final concentration of forward and reverse primers) were used to check the expression of respective genes with a 1× final concentration of SYBR Green PCR master mix (SYBR Green I Dye, AmpliTaq Gold DNA polymerase, dNTPs with UTP, passive reference, and optimized buffer components), 0.25 U/ml multiscribe reverse transcriptase, 0.4 U/ml of RNase inhibitor, and 10 ng of tissue RNA in a 50-μl PCR mixture according to the manufacturer’s protocol (Applied Biosystems). The temperature profile included an initial 30 min at 48°C (for cDNA synthesis) and then denaturation at 95°C for 10 min to deactivate the reverse transcriptase and activate the thermoscript taq polymerase. This was immediately followed by 40 cycles of denaturation at 95°C for 15 s, 60 s at 60°C annealing, and elongation with optics on for fluorescence monitoring. The specificity and purity of the amplification reaction was determined by performing a melting-curve analysis. The relative quantification of gene expression by real-time RT-PCR in a sample was determined by comparing the target-amplified product against GAPDH (internal standard) within the same sample. GAPDH mRNA expression was not significantly different among the various groups. Treated-to-control ratios were tested for deviation from unity by calculation of confidence limits.

Statistical Analysis

All values were expressed as means ± SE. Results in Figs. 3 and 4 were analyzed by a repeated-measures ANOVA across time post-MI and time before death and planned pairwise comparisons (comparing 30-mg dose with other conditions). One-way ANOVA and Dunnett’s tests were also used in Fig. 4A to compare mean arterial pressure (MAP) before treatment and MI procedure. Figure 5 was analyzed by a two-way repeated-measures ANOVA across

Fig. 1. Flow diagram of the various groups of rats according to treatment and the experimental design. MI, myocardial infarction.
volume by dosage and condition (large MI vs. sham). Finally, results within a given MI group and across treatment groups in Fig. 6, and Tables 1–3 were analyzed by a one-way ANOVA and Dunnett’s test. 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

In the control group ($n = 190$), 91 of 147 rats with a large MI died within the first 24 h (38.1% survival) and 16 died over the following 37 days, leaving 40 survivors (27.2%) at the end of the 38-day protocol. Of the rats receiving losartan at 3 mg·kg$^{-1}$·day$^{-1}$ ($n = 161$), 78 of 123 with a large MI died within the first 24 h (36.6% survival) and 4 died over the following 37 days, leaving 41 survivors (33.3%). Of the rats receiving losartan at 30 mg·kg$^{-1}$·day$^{-1}$ ($n = 103$), 64 of 82 with a large MI died within 24 h (21.9% survival, $P < 0.01$ vs. control and $P < 0.04$ vs. low-dose losartan). Of the remaining 18 rats, 7 died over the following 38 days, leaving only 11 survivors (13.4%, $P < 0.0001$ vs. control and $P < 0.0001$ vs. low-dose losartan) (Fig. 2A).

If one considers only rats with a large MI that survived the first 24 h post-MI, over the following 37 days, the group receiving low-dose losartan with a gradual increase in dose had the best survival, 91.5% compared with 71.4% in the control group and 61.1% in high-dose losartan ($P < 0.02$ and $P < 0.01$, respectively) (Fig. 2B).

24-h Protocol

Incidence and duration of ventricular arrhythmias. No ventricular tachycardia or ventricular fibrillation was recorded during the 6-h baseline recording before coronary artery occlusion (Fig. 3). There was no difference in average ventricular arrhythmia score between large MI control rats and large MI rats receiving high-dose or low-dose losartan.

Ambulatory blood pressure. MAP did not differ among the three treatment groups before losartan administration, but it was significantly lower after 7 days of losartan treatment in the 30-mg·kg$^{-1}$·day$^{-1}$ group (Fig. 4A). MAP decreased as a result of a large MI in all three groups. Over the 24-h post-MI period, MAP differed significantly across treatment groups. Planned pairwise comparisons revealed that following a large MI, high-dose losartan-treated animals had a lower MAP compared with control and low-dose-treated groups ($P = 0.05$ and 0.02, respectively). After a large MI, the MAP of rats receiving 3 mg·kg$^{-1}$·day$^{-1}$ losartan did not significantly differ from those of control rats.

In high-dose losartan-treated rats with a large MI that died in the first 24-h post-MI, MAP was extremely low before death. Compared with controls and low-dose losartan-treated rats with equivalent MI size, MAP was lower over the 60- to 5-min time interval before death (both $P < 0.05$) (Fig. 4B), suggesting that hypotension played a role in the death.

38-Day Protocol

Echocardiographic measurements. In the sham-operated groups, losartan at a low or high dose did not have a discernable effect on echocardiographic measurements (Table 1). In control large MI, there was significant LV dilatation with LV circumference and area in systole and diastole all increasing compared with sham controls. There was also significant thinning of both the anterior and posterior walls, an increase in LV diastolic and systolic dimensions (LVDD and LVSD, respectively) and impaired LV function as reflected by decreased LV shortening fraction (LVSF) and LV ejection fraction (LVEF).

Losartan at high and low doses had similar effects. After a large MI, the doses attenuated LV dilatation and helped preserve LV function compared with control large MI.

Hemodynamic effects of chronic losartan treatment. Thirty-eight days post-MI, systolic and diastolic blood pressures were lower (Table 2) in sham-operated, losartan treated groups (both were receiving 30 mg·kg$^{-1}$·day$^{-1}$ at death) as compared with control sham-operated rats.
As compared with their sham counterparts, control large MI rats had a decrease in LV systolic pressure (LVSP), an increase in LV end-diastolic pressure (LVEDP), and a decrease in all measured indexes of contractility and relaxation. These changes were accompanied by an increase in RV systolic pressure (RVSP) and RV end-diastolic pressure (RVEDP).

Both groups of losartan-treated large MI rats had similar hemodynamic changes. Systemic arterial pressure as well as LVSP decreased compared with control animals with a large MI. LVEDP, RVSP, and RVEDP all increased less, suggesting improved LV function. LV \( \frac{dP}{dt} \), when corrected for LVSP, also improved compared with control large MI.

**Morphological measurements.** In sham-operated rats, losartan treatment had little effect. The only change was a reduction in LV mass in the high-dose losartan-treated group (Table 3). Compared with their sham counterparts, control large MI rats had a significant increase in RV, atrial, and lung weights. In the two large MI losartan-treated groups, the increase in RV, septal, atrial, and lung weights were less marked than in control large MI.

**Passive LV pressure-volume relationship.** Losartan treatment exerted a significant effect on the passive pressure-volume relationship \( (P < 0.02) \). In sham-operated rats, losartan caused a slight leftward shift in the passive pressure-volume relationship. A rightward shift in the passive pressure-volume relationships of all large MI groups was observed; however, the rightward shift was less in the losartan-treated rats (Fig. 5).

**MI size.** Animals with a large infarct surviving the 38-day protocol used for real-time PCR had a similar infarct surface area in the high-dose group \( (1.05 \pm 0.06 \, \text{cm}^2, \ n = 11) \) compared with \( 1.21 \pm 0.04 \, \text{cm}^2 \) \( (n = 31) \) and \( 1.24 \pm 0.05 \, \text{cm}^2 \) \( (n = 14) \) in the nontreated and low-dose losartan-treated animals, respectively \( (P > 0.05) \). No difference in MI size was also observed in animals who underwent passive LV pressure-volume assessment and had their MI size calculated via HPS staining and computer planimetry in the nontreated \( (n = 9) \) and low-dose losartan-treated \( (n = 27) \) groups \( (45.1 \pm 1.8\% \) vs. \( 41.6 \pm 1.4\% \) of the LV wall circumference, respectively, \( P = 0.2) \).

**Real-time PCR.** Losartan had no effect on LV target mRNA expression in sham-operated rats (Fig. 6). In the control large MI groups, there was a significant reduction in GLUT-4 mRNA expression and an increase in fetal gene expression [skeletal \( \alpha \)-actin (SKACT), atrial natriuretic factor (ANF), and \( \beta \)-myosin heavy chain mRNA] compared with sham-operated rats. Both losartan-treated large MI groups had significantly higher levels of GLUT-4 mRNA expression compared with the control large MI groups, and LV SKACT, ANF, and \( \beta \)-MHC mRNA expression were significantly less elevated than the control large MI groups.

**DISCUSSION**

The main findings of this study are that 1) the administration of the ARB losartan in the pre-, peri-, and post-large MI period does not result in improved 38-day post-MI survival and that the administration of high doses in the peri-MI period actually increases mortality due to excessive hypotension; 2) losartan also does not reduce peri-MI (24 h) arrhythmias in a meaning-
ful way; and 3) losartan has beneficial effects on LV function remodeling and fetal gene expression, and that this results in improved survival starting 24 h post-MI. Thus, during the peri-MI period, as has been found with ACEi in the clinical setting, large doses of ARBs should be avoided. Rather, ARBs should be given at lower doses early post-MI and increased progressively over time. A similar progressive dosing schedule of ARBs in the peri-MI and early post-MI settings should form the basis of the administration of ARBs in further experimental studies in the field.

Peri-MI Effects of Losartan: Concerns Regarding Hypotension

In animals with a large MI, higher dose of losartan given peri-MI resulted in an increase in 24-h post-MI mortality. Careful analysis of MAP in rats dying during the first 24 h post-MI revealed that rats receiving the higher dose of losartan more frequently had marked hypotension the hour before death, suggesting that hypotension contributed to their death. In these rats, within 1 h of dying, MAP ranged between 50 and 60 mm Hg, much lower than the 75- to 90-mm Hg in rats dying in the control and lower dose losartan-treated groups, suggesting a possible relationship between the hypotension caused by losartan and increased mortality. Persistent hypotension after MI is a serious concern, because it has been linked to the occurrence of tachyarrhythmias and ventricular fibrillation leading to sudden death (8). It must nevertheless be noted that the lower dose of losartan used in this study resulted in partial AT1 receptor blockade as reflected by the rightward shift in ANG II dose-response curve that it caused. In the CONSENSUS II trial, a high dose of the ACEi enalapril started within the first 24 h after an acute MI resulted in a significantly higher incidence of hypotension and a trend toward increased mortality within 6 mo following the initial infarction (26). Worrisome hypotension has also been reported in other clinical trials where ACE inhibition was initiated within the first day of an MI (1, 7a).

The idea of delaying therapy to reduce the onset of hypotension is a controversial one given that infarct expansion and remodeling is initiated within hours of an MI. In favor of an early aggressive approach is the HEART study by Pfeffer et al. (20), in which patients were randomized to early (within 24 h) low-dose ramipril, early higher dose (progressive dose increase), and delayed ramipril use. The low dose of ramipril chosen for this study did not normally have hemodynamic effects but did have mild hemodynamic effects in the acute MI setting (20). Of the three treatment groups, the early high-dose group had the best effects on LV remodeling, suggesting that adequate doses of an ACEi early post-MI is important. In the present study, the initial dose of losartan used (3 mg·kg⁻¹·day⁻¹) was proportionately greater than the dose used in the HEART study. In pilot studies, we showed this dose to cause a significant rightward shift of the ANG II dose-response relationship, and in a previous study, this dose has been shown to have significant beneficial effects in the post-MI setting (4). The dose was then rapidly increased to 10 mg·kg⁻¹·day⁻¹, a larger dose also shown to be useful after an MI (12), and finally to 30 mg·kg⁻¹·day⁻¹. The results of the present study thus support the early use of lower doses of an ARB after an MI with up titration to full doses early, as the HEART study did in with ACEi.

Table 1. Echocardiographic measurements at 30 days post-MI

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Ant W, cm</th>
<th>LVDD, cm</th>
<th>Pos W, cm</th>
<th>LVSD, cm</th>
<th>LVSF, %</th>
<th>LVEF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>20</td>
<td>0.2±0.02</td>
<td>0.8±0.02</td>
<td>0.2±0.01</td>
<td>0.6±0.04</td>
<td>31±2</td>
<td>46±4</td>
</tr>
<tr>
<td>Sham + losartan (30 mg·kg⁻¹·day⁻¹)</td>
<td>10</td>
<td>0.2±0.01</td>
<td>0.8±0.04</td>
<td>0.1±0.01</td>
<td>0.6±0.05</td>
<td>29±3</td>
<td>39±4</td>
</tr>
<tr>
<td>Sham + losartan (3 mg·kg⁻¹·day⁻¹)</td>
<td>16</td>
<td>0.14±0.01</td>
<td>0.8±0.03</td>
<td>0.2±0.01</td>
<td>0.6±0.04</td>
<td>29±3</td>
<td>41±4</td>
</tr>
<tr>
<td>Large MI</td>
<td>40</td>
<td>0.1±0.01*</td>
<td>1.14±0.01*</td>
<td>0.1±0.00</td>
<td>1.1±0.01*</td>
<td>8.1±0.6*</td>
<td>16±1.0*</td>
</tr>
<tr>
<td>Large MI + losartan (30 mg·kg⁻¹·day⁻¹)</td>
<td>11</td>
<td>0.1±0.01*</td>
<td>1.09±0.01†</td>
<td>0.14±0.01*</td>
<td>0.95±0.03†</td>
<td>12.4±2†</td>
<td>21±3*</td>
</tr>
<tr>
<td>Large MI + losartan (3 mg·kg⁻¹·day⁻¹)</td>
<td>41</td>
<td>0.1±0.01*</td>
<td>1.11±0.01*</td>
<td>0.12±0.01*</td>
<td>0.99±0.01†</td>
<td>11±0.7†</td>
<td>20±1.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. MI, myocardial infarction; Ant W, anterior wall thickness; LVDD, left ventricular (LV) diastolic dimension; Pos W, posterior wall thickness; LVSD, LV systolic dimension in short axis, just below plane of mitral valve; LVSF, LV shortening fraction; LVEF, LV ejection fraction. *P < 0.05 vs. sham; †P < 0.05 vs. control large MI.

Table 2. Hemodynamic measurements at 38 days post-MI

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>HR, beats/min</th>
<th>RVSP, mmHg</th>
<th>RVEDP, mmHg</th>
<th>+dP/dt/dr RVSP</th>
<th>LVSP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>+dP/dt/dr LVSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>15</td>
<td>123±4</td>
<td>92±2</td>
<td>274±14</td>
<td>28±1</td>
<td>2±0.5</td>
<td>69±3</td>
<td>120±5</td>
<td>4±1</td>
<td>70±3</td>
</tr>
<tr>
<td>Losartan (30 mg·kg⁻¹·day⁻¹)</td>
<td>9</td>
<td>104±4†</td>
<td>78±4†</td>
<td>303±25</td>
<td>26±1</td>
<td>1±1</td>
<td>80±6</td>
<td>101±3†</td>
<td>3±0.5</td>
<td>73±4</td>
</tr>
<tr>
<td>Losartan (3 mg·kg⁻¹·day⁻¹)</td>
<td>16</td>
<td>100±3†</td>
<td>75±3†</td>
<td>284±10</td>
<td>26±1</td>
<td>1±0.5</td>
<td>69±2</td>
<td>99±3†</td>
<td>6±1</td>
<td>73±2</td>
</tr>
<tr>
<td>Large MI</td>
<td>37</td>
<td>102±3*</td>
<td>81±2*</td>
<td>289±8</td>
<td>43±2*</td>
<td>5±1</td>
<td>53±2*</td>
<td>97±3*</td>
<td>15±1*</td>
<td>57±1*</td>
</tr>
<tr>
<td>Losartan (30 mg·kg⁻¹·day⁻¹)</td>
<td>11</td>
<td>96±5*</td>
<td>73±3</td>
<td>273±27</td>
<td>32±4†</td>
<td>2±0.5†</td>
<td>63±3†</td>
<td>93±4</td>
<td>10±2*</td>
<td>56±2*</td>
</tr>
<tr>
<td>Losartan (3 mg·kg⁻¹·day⁻¹)</td>
<td>41</td>
<td>89±3.9†</td>
<td>69±2†</td>
<td>301±13</td>
<td>31±1†</td>
<td>2±0.3†</td>
<td>62±2†</td>
<td>83±3†</td>
<td>10±1†</td>
<td>59±2†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; RVSP, right ventricular (RV) systolic pressure; RVEDP, RV end-diastolic pressure; +dP/dt, pressure change over time; LVSP, LV systolic pressure; LVEDP, LV end-diastolic pressure. *P < 0.05 vs. sham; †P < 0.05 vs. control large MI.
The findings of this study contrast previous reports that have demonstrated beneficial outcomes with the use of ARBs administered pre- or early post-MI at doses similar to or higher than the 30-mg·kg\(^{-1}\)·day\(^{-1}\) used in this study. In a study by Zhu et al. (28), the authors reported that pretreatment with losartan at 40 mg·kg\(^{-1}\)·day\(^{-1}\) for 10 wk, followed by 17 min of ischemia and 2 h of reperfusion (IR), resulted in decreased ventricular arrhythmias, decreased MI size, and decreased 2 h of ischemia and 2 h of reperfusion (IR), resulted in decreased hemodynamic modification with this high dose of losartan. The explanation for these differences is not clear but may have resulted from a combination of the experimental protocol (IR), the short followup period, and the anesthesia used in their study. Our rats were awake and mobile most of the 24-h followup period. In another study, Ruzicka et al. (23), who started losartan treatment (15 mg·kg\(^{-1}\)) for 10 wk, followed by 17 min of ischemia and 2 h of reperfusion (IR), resulted in decreased ventricular arrhythmias, decreased MI size, and decreased 2 h of reperfusion (IR). Results from Zhu et al. (28) would suggest that pretreatment with losartan at 40 mg·kg\(^{-1}\)·day\(^{-1}\) decreases ventricular arrhythmias in a rat model of IR. In another study (27), the same group found that longer duration of pretreatment (i.e., 4 wk) was more cardioprotective than shorter periods (i.e., 1 wk and 1 day) of pretreatment before IR in rats, suggesting that longer pretreatment in our study may have resulted in different findings. Alternatively, it may be that losartan effects the arrhythmogenic properties in IR differently from its effects in transmural MI, the mechanisms involved in both being quite different (17). It may also be that the lack of hypotension associated with IR combined with high doses of losartan is both necessary for its antiarrhythmic effects to occur. In the study by Zhu et al. (28), despite large doses, losartan did not modify systolic blood pressure throughout the study, whereas in our study, the hypotension associated with the MI and high-dose losartan may have negated its antiarrhythmic effects.

**Beneficial Post-MI Effects of Losartan**

Losartan treatment in rats surviving the acute (24-h) MI period with a low dose, followed by timed, gradual increase in the dosing regimen, resulted in dramatic improvement in survival over the next 37 days of the protocol. This improvement in survival was accompanied by an improvement in LV remodeling and function, a finding compatible with previous experimental post-MI studies using ARBs in rats (4, 13, 16, 24, 25), and more importantly, compatible with the results of the VALIANT study (21) that found the ARB valsartan to be as effective as the ACEi captopril post large MI. Animals in both high and progressive dose groups that survived the 38-day protocol also presented similar improvements in LV remodeling and function reported by others, suggesting that in survivors both dosing regimens are equally cardioprotective. Our findings are supported by a previous study by Nakamura et al. (18), who demonstrated that chronic low- and high-dose ARB treatment following an MI in rats is equally beneficial in terms of LV remodeling and function. In our study, the observed improvement in LV remodeling and function was accompanied by attenuation in fetal gene reexpression and in preservation of the cardiac expression of the glucose transporters GLUT-4. Our study confirms the results of Hanatani et al. (9) who have previously reported attenuation of fetal gene expression in a shorter post-MI study with another ARB in the post-MI setting. This is, however, the first report of preservation of the glucose transporter GLUT-4 in the post-MI setting. Greater expression of GLUT-4 could result in improved glu-

### Table 3. Morphological measurements at 38 days post-MI

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>LV Total, g</th>
<th>Septum, g</th>
<th>RV, g</th>
<th>Scar, g</th>
<th>Atria, g</th>
<th>Lungs, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>430±9</td>
<td>0.83±0.03</td>
<td>0.27±0.02</td>
<td>0.25±0.01</td>
<td>0.1±0.01</td>
<td>1.6±0.05</td>
<td></td>
</tr>
<tr>
<td>Losartan (30 mg·kg(^{-1})·day(^{-1}))</td>
<td>9</td>
<td>420±10</td>
<td>0.70±0.03†</td>
<td>0.24±0.02</td>
<td>0.22±0.01</td>
<td>0.07±0.003</td>
<td>1.6±0.04</td>
<td></td>
</tr>
<tr>
<td>Losartan (progressive)</td>
<td>6</td>
<td>448±10</td>
<td>0.77±0.04</td>
<td>0.22±0.02</td>
<td>0.25±0.02</td>
<td>0.08±0.01</td>
<td>1.9±0.17</td>
<td></td>
</tr>
<tr>
<td>Large MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>422±8</td>
<td>0.67±0.02</td>
<td>0.31±0.01</td>
<td>0.42±0.02*</td>
<td>0.1±0.01</td>
<td>0.20±0.01*</td>
<td>2.8±0.12*</td>
</tr>
<tr>
<td>Losartan (30 mg·kg(^{-1})·day(^{-1}))</td>
<td>11</td>
<td>411±12</td>
<td>0.65±0.02*</td>
<td>0.28±0.01</td>
<td>0.29±0.03†</td>
<td>0.1±0.01</td>
<td>0.12±0.01†</td>
<td>2.0±0.24†</td>
</tr>
<tr>
<td>Losartan (progressive)</td>
<td>14</td>
<td>441±7</td>
<td>0.64±0.03†</td>
<td>0.26±0.01†</td>
<td>0.32±0.03†</td>
<td>0.1±0.01</td>
<td>0.16±0.01†</td>
<td>2.2±0.11†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. BW, body weight; LV total, LV + septum. *P < 0.05 vs. sham; †P < 0.05 vs. control large MI.
cose utilization in the failing heart and thus improved cardiac energetics.

We also documented a significant improvement in right ventricular function as well as a reduction in pulmonary congestion. Zornoff et al. (29) have reported that RV dysfunction is a very important predictor of survival. Thus beneficial effects of losartan on RV hypertrophy, pressure, and function could at least partially be responsible for the observed improvement in survival in our study.

Despite these improvements, we were unable to show a reduction in infarct size with losartan pre- and post-MI treatment, which has been previously reported by Sladek et al. (25). Once again our findings contrasts with the findings of Zhu et al. (28), which demonstrated that pretreatment with 40 mg·kg⁻¹·day⁻¹ of losartan decreased MI size. But again the results of Zhu et al. (28) were in the setting of 17 min of ischemia and then 2 h of reperfusion, a setting in which they found no reduction in systolic blood pressure with losartan.

This is the first time that the pre- and peri-MI effects of losartan have been combined with their long-term post-MI effects. Why the combined peri- and post-MI use of losartan did not result in improved survival is not clear, but an early detrimental effect of losartan, even in the smaller dose, cannot be excluded. Alternatively, because no early harm could be documented acutely (<24 h) with the lower dose, this lack of overall benefit may have resulted from inadequate sample size and power to detect a difference. A study by Hu et al. (11) has also reported that losartan at 10 mg·kg⁻¹·day⁻¹ started early post-MI did not attenuate structural dilatation following coronary occlusion in rats. At this time, no other study evaluating the peri-MI effects of an ARB exists to evaluate consistency with our results.

**Limitations**

Animals were anesthetized at the time of surgery. This may have influenced arrhythmias and survival in the first 2 h post-MI. Thereafter rats were awake and unrestrained for the next 22 h of monitoring. A number of rats died during the study, which may have influenced hemodynamic measurements at the end of the study. To minimize problems related to the loss of rats during the study, we classified rats according to MI size. Finally, because we did not include a low-dose group without progressive uptitration, it is possible that it was unnecessary to progressively increase the dose. This does not, however, change the clinical implications of our findings that high doses of an ARB should be avoided early after a MI because they result in excessive hypotension and death. The combined results of the OPTIMAAL and VALIANT studies have established the need to uptitrate post-MI.

In conclusion, this study indicates that pre- and peri-MI treatment with the ARB losartan exerts no beneficial effect on survival and ventricular arrhythmias in the acute period post-MI, and high doses may even be detrimental due to excessive hypotension. Losartan does not have early antiarrhythmic properties, but, when given in progressively increasing doses, it results in improved LV remodeling and function, which in 24-h post-MI survivors, results in improved survival, reduced fetal gene expression, and preserved cardiac GLUT-4 expression despite no difference in infarct size.

**ACKNOWLEDGMENTS**

The assistance of Aurora Mendelsohn on the statistical analysis of this study is gratefully acknowledged.
REFERENCES


