Effects of angiotensin II receptor blockade versus angiotensin-converting-enzyme inhibition on ventricular remodelling following myocardial infarction in the mouse

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ABSTRACT

Left ventricular (LV) remodelling following myocardial infarction (MI) is associated with increased morbidity and mortality. Previous data suggest that angiotensin II (Ang II) plays a central role in the molecular events contributing to LV remodelling. We explored the effects of angiotensin-converting-enzyme (ACE) inhibition versus Ang II (AT₁) receptor blockade on LV remodelling in mice post-MI. Mice underwent sham procedure or left coronary artery ligation, and received placebo, the AT₁ receptor antagonist, losartan or the ACE inhibitor, enalapril. At 6 weeks, echocardiography and haemodynamic studies were performed. Infarct size and interstitial collagen content were determined. Expression of genes encoding atrial natriuretic peptide (ANP), collagen type I, AT₁a and AT₁b receptors were measured. The placebo MI group showed increased LV end-diastolic diameter, LV end-systolic diameter with depressed fractional shortening (P < 0.01 versus shams), increased LV mass and volume (both P < 0.01 versus shams). The placebo MI group also exhibited increased non-infarct zone collagen content (P < 0.01), ANP (P < 0.01) and collagen type I (P < 0.01) gene expression, with a non-significant rise in AT₁a receptor gene expression. Neither losartan or enalapril prevented LV dilation or improved fractional shortening. Both similarly lowered systolic blood pressure (P < 0.01 for each versus placebo). Losartan and enalapril inhibited LV hypertrophy (P < 0.01), and decreased ANP (P < 0.01) and collagen type I gene expression (P < 0.05). Levels of AT₁a receptor gene expression were higher than shams (P < 0.05 for both), but similar to placebo. AT₁b receptor gene expression was much lower than that for AT₁a receptor and similar in all groups. Thus, in this model, AT₁ receptor antagonism and ACE inhibition have equivalent inhibitory effects on myocardial hypertrophy and fibrosis. These results serve as an important basis for planned investigations to evaluate the anti-remodelling effects of these agents on mice in which genetic manipulations are used to disrupt components of the Ang II signalling system.

Key words: angiotensin-converting-enzyme inhibitors, angiotensin II, myocardial infarction, gene expression, mouse, ventricular remodelling.

Abbreviations: ACE, angiotensin-converting enzyme; Ang, angiotensin; AT receptor, Ang II receptor; ANP, atrial natriuretic peptide; BM, body mass; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LV, left ventricular; MI, myocardial infarction; SBP, systolic blood pressure.

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INTRODUCTION

Left ventricular (LV) remodelling following myocardial infarction (MI) is characterized by progressive increases in LV chamber size and sphericity [1,2]. Following a large MI, LV dilatation occurs gradually over the course of months to years, resulting primarily from eccentric hypertrophy of the non-infarcted myocardium [3]. Though LV remodelling may serve to maintain stroke volume [4], progressive LV dilatation is associated with an increased incidence of heart failure and death [2], supporting the theory that LV remodelling following MI is a maladaptive response.

Clinical trials have demonstrated that angiotensin-converting-enzyme (ACE) inhibitors reduce the extent of LV chamber enlargement in patients with LV dysfunction following MI [5,6]. Accordingly, ACE inhibitors have been shown to reduce morbidity and mortality post-MI [5,7–9]. The mechanism of benefit is thought to be multifactorial, including reduction of angiotensin (Ang) II formation therefore reducing activation of Ang II receptors. ACE also possesses important kininase activity, and ACE inhibitors therefore increase local bradykinin levels, which may contribute to the observed anti-remodelling effects in some animal models [10].

To date, three Ang II receptors have been described: type I, type II, and type IV (denoted AT₁, AT₂, and AT₄ respectively). Activation of the AT₁ receptor accounts for most of the known cardiovascular effects of Ang II, such as vasoconstriction and cellular growth stimulation. Within the myocardium of multiple species, Ang II formation may also result from the activity of proteases other than ACE, such as chymase [11]. For this reason, non-peptide antagonists of the AT₁ receptor have gained clinical interest, because these agents block AT₁ receptor activation, regardless of the mechanism by which Ang II is formed.

Previous studies comparing the effects of ACE inhibition versus AT₁ receptor blockade on LV remodelling have shown disparate results among species [12–15]. These observations may result from the wide species variability of myocardial levels of Ang II, its receptors and other components important to Ang II signalling [11,16]. We previously developed a MI model in mice to investigate the mechanisms underlying the pathophysiology of post-MI ventricular remodelling in normal and transgenic mice [17]. The present study has compared the effects of ACE inhibition versus AT₁ receptor blockade on the morphometric and gene-expression markers of post-MI ventricular remodelling in a mouse MI model.

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METHODS

Animals

Sixty-five male C57BL/6J (Charles River, Wilmington, MA, U.S.A.) mice, 8–10 weeks old, with a mass of 20–25 g, were studied. Mice were housed at no more than five per cage at an animal facility (Association for the Assessment and Accreditation of Laboratory Animal Care approved) with 12-h light/dark cycles and given free access to standard rodent chow (ProLab chow; Agway, Syracuse, NY, U.S.A.) and water. This protocol was approved by the Institutional Animal Research Committee.

Preliminary dose-finding study

To assure equivalent effects of drug treatment, preliminary dose-finding studies were performed using normal C57BL/6J mice. Mice (n = 5 for each dosing group) were randomized to a range of doses of enalapril (50–200 mg/l drinking water), losartan (200–600 mg/l drinking water) or placebo. Seven days later, mice were anaesthetized and underwent right carotid artery catheterization to measure systolic blood pressure (SBP). Next, Ang I (for enalapril) or Ang II (for losartan) was administered intravenously via the right external jugular vein in increasing doses ranging from 0.02–200 ng, and SBP was measured 60 s after each dose. Enalapril at a concentration of 150 mg/l in the drinking water, and losartan at a concentration of 600 mg/l, resulted in equivalent lowering of SBP compared with placebo (enalapril, 83 ± 13 mmHg; losartan, 83 ± 14 mmHg; P < 0.05 each compared with placebo, 99 ± 16 mmHg). These doses also caused equivalent shifts in the dose–response curves to Ang I for enalapril and Ang II for losartan (Figure 1).

Left coronary ligation

Study animals were randomized to MI versus sham operation. MI was induced in mice as described previously [17]. Briefly, mice were anaesthetized using a mixture of ketamine and pentobarbital via intraperitoneal injection, intubated and then ventilated with a small animal respirator (Harvard Apparatus, Holliston, MA, U.S.A.). A left thoracotomy was performed; MI was induced by placing a suture through the myocardium in the anterolateral LV wall, interrupting left coronary artery flow. For animals undergoing a sham operation, a ligature was placed in an identical location but not tied. The chest cavity was then closed in layers and the animal was allowed to recover. Mice were then randomized to placebo, losartan or enalapril administered immediately post-MI in the drinking water for 6 weeks.

Echocardiography

Six weeks following surgery, all mice underwent trans-thoracic echocardiographic evaluation as described pre-
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Previously [17]. A commercially available echocardiography system (HDI 3000; ATL Ultrasound, Bothell, WA, U.S.A.) was utilized with a dynamically focused 10 MHz linear array transducer using a depth setting of 0.5–1.0 cm. Animals were anaesthetized with ketamine and xylazine, weighed, and both two-dimensional images and M-mode tracings (sweep speed 50–100 mm/s) were then recorded from the short axis view at the papillary muscle level [17].

Using M-mode tracings, LV end-diastolic diameter (EDD) and end-systolic diameter (ESD) were measured to the nearest 0.1 mm, averaging three cardiac cycles. Fractional shortening (FS; percentage) was calculated using the standard equation [17]:

\[
FS = \frac{(LVEDD - LVESD)}{LVEDD} \times 100
\]

All echocardiographic measurements were performed by an observer blinded to the treatment groups.

Haemodynamic evaluation
At the conclusion of the echocardiographic studies, animals underwent full haemodynamic study as described previously [17]. The right external jugular vein was cannulated with silastic tubing. The right carotid artery was isolated and ligated distally. A segment of flame-stretched Teflon tubing (originally 0.102 cm) was inserted and secured in place. After allowing 2 min for haemodynamic stabilization, systemic arterial pressure was recorded using the MacLab 4 s computerized recording and analysis system (AD Instruments, Milford, MA, U.S.A.).

Left ventricular pressure
The animal was then intubated and a left thoracotomy was performed. The LV apex was punctured using a 25-gauge fluid-filled needle attached to a Transpac II pressure transducer (Abbott Laboratories, Chicago, IL, U.S.A.). LV pressure and its first derivative were recorded. While under anaesthesia, the animal was killed with a 0.3 ml injection of 1 M KCl via the jugular venous catheter, arresting the heart in diastole [17].

Passive pressure–volume relationships
Following post mortem, passive pressure–volume relationships were obtained as described previously [17]. From the passive pressure–volume data, log transformed curves were derived, and the resultant linear relationship was used to derive the constant, \( k \) (slope), by the linear regression equation:

\[
\ln P = k \times V + c,
\]

where \( V \) is the volume, \( P \) the pressure and \( c \) the intercept on the \( y \)-axis [18]. LV volume at a common distending pressure (10 mmHg) was calculated from this regression equation. All haemodynamic data subsequently were analysed by an observer blinded to treatment groups. Next, the heart was excised, and the right ventricle free wall and LV (with septum intact) were weighed to the nearest 0.1 mg. Using a scalpel, the LV base was carefully trimmed to remove any remaining atrial tissue, and a 1 mm segment of the base was removed, immediately snap frozen in liquid N\(_2\), and stored at \(-70^\circ\)C for future RNA analysis. The remaining portion of the LV was immersed in 10% buffered formalin. After 24–48 h of fixation, the LV was sectioned transversely into three equal segments from apex to base and embedded in paraffin.

Morphometric evaluation
Serial sections (5 \( \mu \)m) were prepared using a standard microtome. Sections were mounted and stained with haematoxylin and eosin for determination of infarct size. Quantitative histological analyses were performed on a computerized image analysis system using the public domain image analysis system ImageJ.
domain National Institutes of Health Image program (http://rsb.info.nih.gov/nih-image/). Infarct size was determined as described previously [17]. Collagen content within the non-infarct zone was measured using a modification of a previously described method [12]. Histological images from Sirius Red-stained sections were captured at a magnification of ×100. Two LV sections per animal were analysed. From each section, four images each from the sub-endocardium, mid-myocardium and sub-epicardium were obtained giving a total of 24 images per heart. Using the computerized image analysis system, collagen content was measured by planimetry of the area occupied by collagen, expressed as the percentage of total tissue area.

**Semi-quantitative reverse-transcription PCR**

Total cellular RNA was isolated using the Trizol reagent (Gibco), according to the manufacturer's instructions, from each frozen LV segment obtained at the time when the animal was killed. RNA integrity was confirmed by visualization of distinct 18 S and 28 S bands after electrophoresis on 1.5% agarose gels stained with ethidium bromide. To remove contaminating genomic DNA, 1 μg of RNA was treated with Rnase free Dnase (RQ1, Promega) and then reverse transcribed using Moloney-murine-leukaemia virus reverse transcriptase (Gibco). Duplicate RNA samples were incubated in the same buffer with no reverse transcriptase added to act as negative controls. PCR was performed using 2.5 units of Taq polymerase (Gibco). For a comparative internal standard, primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were added to each PCR reaction mixture. For atrial natriuretic peptide (ANP), collagen type I, and AT₁b receptor PCR, GAPDH primers included sense, 5'-TGTTCCAGTAGTCCTCAA-CTCACGG-3', and antisense, 5'-GGCCCCTCCTGTTATTATGG-3', yielding a 714-bp product. Cycling parameters included 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 60 s for 30 cycles. To measure AT₁b receptor gene expression, a high-fidelity Taq polymerase with anti-Taq polymerase antibody (Platinum Taq; Gibco) was used because of the low abundance of AT₁b receptor message. The sense primer was 5'-ATTCAGTTTCTGTGATGTC-3', and antisense, 5'-TCCACTTCAAAACAATACGC-3', resulting in a 303-bp fragment. Cycling parameters included 94 °C for 30 s, annealing at 48 °C for 30 s, and extension at 68 °C for 60 s for 36 cycles. Because of the much greater relative amount of GAPDH mRNA, an aliquot of the PCR reaction was removed after 27 cycles to normalize measurements for AT₁b receptor gene expression. To assure that the number of cycles chosen for each PCR analysis was along the linear phase of the reaction, varying amounts of RNA (0.5–2.5 μg) were reverse transcribed and subjected to the PCR as described. The amount of cDNA product for all mRNAs analysed increased linearly with increasing amounts of input RNA (results not shown). For a given gene, PCR experiments were performed 2–3 times for each sample, yielding highly reproducible results (correlation coefficients between experiments ≥ 0.9).

All reverse-transcription samples were run on 1–2% agarose gels stained with ethidium bromide and visualized under UV light. A digital image of the illuminated gel was obtained and the amount of a given PCR product was quantified by densitometric scanning using a commercially available system (Alpha Innotec, San Leandro, CA, U.S.A.). All products were expressed as a ratio to GAPDH. The mean value of the sham group was assigned an arbitrary value of 1 for each analysis.

**Statistical analysis**

All results are expressed as the means ± S.E.M. All four groups (sham, placebo, losartan and enalapril) were compared by ANOVA using the post-hoc Student-Newman-Keuls method for multiple group comparison. Survival curves were constructed using Kaplan-Meier estimate; the curves pertaining to each treatment group were compared with the placebo MI group using the log-rank test.

**RESULTS**

**Survival**

Sham operation was performed in twelve mice, all of whom survived. Fifty-three mice underwent left coronary ligation. Two animals died during surgery; thus, 17 mice were randomized to enalapril, losartan or placebo. One animal in each of the losartan and enalapril groups had no
Figure 2 Kaplan-Meier survival curves for infarct groups
Survival in the losartan group was significantly improved compared with placebo by the log-rank test ($P < 0.04$).

Table 1 Morphometric and volume data
Results are expressed as the means ± S.E.M. *$P < 0.01$ versus sham; †$P < 0.001$ versus placebo and $P < 0.02$ versus sham. ‡$P < 0.01$ versus sham. RV, right ventricular.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham ($n = 12$)</th>
<th>Placebo ($n = 11$)</th>
<th>Losartan ($n = 14$)</th>
<th>Enalapril ($n = 13$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infarct size (%)</td>
<td>37.3 ± 5.0</td>
<td>44.3 ± 4.3</td>
<td>43.5 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>Infarct size &gt; 50% (n)</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>BM (g)</td>
<td>27.9 ± 2.4</td>
<td>27.6 ± 3.2</td>
<td>27.4 ± 2.0</td>
<td>27.5 ± 1.6</td>
</tr>
<tr>
<td>LV mass/BM (g/kg)</td>
<td>3.11 ± 0.06</td>
<td>3.54 ± 0.18</td>
<td>2.75 ± 0.05†</td>
<td>2.70 ± 0.10†</td>
</tr>
<tr>
<td>RV mass/BM (g/kg)</td>
<td>0.750 ± 0.018</td>
<td>0.849 ± 0.083</td>
<td>0.780 ± 0.023</td>
<td>0.737 ± 0.030</td>
</tr>
<tr>
<td>LV volume ($P = 10$ mmHg)</td>
<td>1.29 ± 0.10</td>
<td>2.78 ± 0.48†</td>
<td>3.09 ± 0.26†</td>
<td>3.05 ± 0.46†</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>3.9 ± 0.1</td>
<td>4.9 ± 0.2†</td>
<td>4.7 ± 0.1†</td>
<td>4.7 ± 0.2†</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>2.7 ± 0.1</td>
<td>3.9 ± 0.3†</td>
<td>4.0 ± 0.2†</td>
<td>3.9 ± 0.2†</td>
</tr>
<tr>
<td>FS (%)</td>
<td>31.6 ± 2.0</td>
<td>20.1 ± 1.8†</td>
<td>16.4 ± 1.6†</td>
<td>16.0 ± 1.5†</td>
</tr>
</tbody>
</table>

evidence of MI by histological analysis, and these two mice were excluded from the analysis. Figure 2 shows survival curves for all three MI groups. Within the placebo group, there were six deaths. Three died within the first week due to cardiac rupture and three died during the subsequent 5 weeks. Autopsies on these three animals revealed no evidence of cardiac rupture. Within the losartan group, two died after the first week post-MI (days 8 and 14); autopsies revealed no evidence of rupture. Within the enalapril group, two mice were killed on day 17: one because of an intra-thoracic infection (autopsy revealed a large MI, infarct size 61%); another was killed because of extensive skin maceration (infarct size 29%). These deaths were included in the survival analyses. An additional mouse within the enalapril group died from cardiac rupture on day 3. Mortality in the losartan group was lower than that in the placebo group ($P < 0.04$). Compared with the placebo group, a trend for improved survival was noted in the enalapril group, but was not statistically significant ($P = 0.18$).

Infarct size, ventricular mass and chamber size at 6 weeks
As shown in Table 1, the enalapril and losartan groups showed mean infarct sizes that were not different to the placebo group. However, a greater number of animals with large infarcts (> 50%) survived in the losartan ($n = 7$) and enalapril ($n = 5$) groups compared with the placebo infarct group ($n = 2$). Body masses among
the infarct and sham groups at the time of killing were similar. The placebo group showed a significant increase in LV mass/body mass (BM) ratio compared with sham group (3.54 ± 0.18 versus 3.11 ± 0.06 g/kg; *P < 0.01). Both losartan and enalapril similarly prevented this increase in LV mass/BM compared with the placebo group (2.75 ± 0.05 and 2.70 ± 0.10 g/kg respectively; †P < 0.001 for each versus placebo). LV mass/BM ratios for the losartan and enalapril groups were also lower than the sham group (†P < 0.02 for both). The placebo group displayed increased mean LV volume (at a common distending pressure of 10 mmHg) of 2.78 ± 0.48 versus 1.29 ± 0.10 ml/kg in the sham-operated controls (†P < 0.01). The mean LV volumes for the losartan and enalapril treated groups were also significantly greater than shams (3.09 ± 0.26 and 3.05 ± 0.46 ml/kg respectively; †P < 0.01 for both), and not different to the placebo group.

**Echocardiographic analysis**

Echocardiographic data obtained at 6 weeks following MI are shown in Table 1. The sham-operated group demonstrated a mean LVEDD of 3.9 ± 0.1 mm and mean LVESD of 2.7 ± 0.1 mm with a fractional shortening of 31.6 ± 2.0%. The placebo MI group developed significant LV dilatation (LVEDD, 4.9 ± 0.2 mm; LVESD, 3.9 ± 0.3 mm) along with a markedly reduced FS compared with the sham group (20.1 ± 1.8%; †P < 0.01 for all values). Animals treated with either losartan or enalapril demonstrated increased mean LVEDD and LVESD and decreased FS values that were not different to those in the placebo MI group.

**Haemodynamics**

Table 2 displays haemodynamics obtained at 6 weeks. Mean heart rates were not different between the sham and placebo group. The mean heart rate in the enalapril group was significantly greater than in each of the other three groups (sham, placebo and losartan groups) (†P < 0.05). SBP in the placebo MI group was lower than that in shams (109 ± 9 versus 123 ± 8 mmHg), but did not reach statistical significance. Both losartan and enalapril significantly reduced SBP (83 ± 4 and 76 ± 3 mmHg respectively; †P < 0.01 versus placebo group, †P < 0.001 versus shams for both). LV end-diastolic pressure was similar among all four groups. Peak positive and negative LV dP/dt values (where P is pressure and t is time) were lower in the placebo group than all the other groups, but these differences did not reach statistical significance.

**Collagen content**

The collagen content (the area of collagen as a percentage of the total tissue area) within the non-infarct zone of the placebo group was significantly increased over that of the shams (3.50 ± 0.28% versus 2.64 ± 0.12% respectively; †P < 0.01). Both the losartan (2.96 ± 0.14%) and enalapril groups (2.96 ± 0.08%) showed non-significant reductions in non-infarct zone collagen content compared with placebo MI groups (P = 0.1 for both).

**Gene expression within the non-infarct zone**

We next examined the effects of AT1 receptor blockade and ACE inhibition on gene expression in the non-infarcted myocardium 6 weeks following MI. The ex-

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**Table 2** Haemodynamic parameters

Results are expressed as the means ± S.E.M. *P < 0.01 versus placebo group, and †P < 0.05 versus sham and losartan; ††P < 0.01 versus placebo, †P < 0.001 versus sham. Bpm, beats per minute; LVEDP, left ventricular end diastolic pressure; LV dP/dt, peak positive or negative first derivative of LV pressure.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (n = 12)</th>
<th>Placebo (n = 11)</th>
<th>Losartan (n = 14)</th>
<th>Enalapril (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>266 ± 16</td>
<td>232 ± 17</td>
<td>274 ± 10</td>
<td>330 ± 24†</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>123 ± 8</td>
<td>109 ± 10</td>
<td>83 ± 4†</td>
<td>76 ± 3‡</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>8.2 ± 0.9</td>
<td>9.6 ± 1.7</td>
<td>8.0 ± 0.9</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>LV + dP/dt (mmHg·s⁻¹)</td>
<td>5289 ± 353</td>
<td>4117 ± 435</td>
<td>4545 ± 477</td>
<td>5009 ± 927</td>
</tr>
<tr>
<td>LV − dP/dt (mmHg·s⁻¹)</td>
<td>3329 ± 247</td>
<td>2315 ± 209</td>
<td>2597 ± 250</td>
<td>3323 ± 548</td>
</tr>
</tbody>
</table>
Enalapril versus losartan following myocardial infarction in mice

Figure 4 Collagen type I and GAPDH gene expression measured by reverse-transcription PCR
Upper panel: examples shown are representative of the treatment groups indicated. Lower panel: results shown are the means ± S.E.M. for the respective groups. *P < 0.01 versus shams; †P < 0.05 versus placebo. Coll, collagen; Enal, enalapril; Los, losartan; Plac, placebo.

expression of the ANP gene increases in concert with and is a marker for myocyte hypertrophy [20]. In the present study, ANP gene expression correlated well with LV mass/BM ratio in the placebo and drug-treated infarct group (r = 0.73, P < 0.0001). As demonstrated in Figure 3, the placebo MI group showed a 9-fold increase in relative ANP gene expression compared with the shams (P < 0.001). Similar to the LV mass data, both losartan and enalapril significantly inhibited the increase in ANP gene expression (P < 0.01 versus placebo group; P not significant versus sham for both).

Within the myocardium, collagen type 1 gene expression has been shown to arise specifically from cardiac fibroblasts [21]. Figure 4 displays the relative amount of collagen type I gene expression in all groups. The placebo MI group demonstrated a 2.5 fold increase in collagen type I gene expression compared with shams (P < 0.01). Treatment with either losartan or enalapril reduced collagen type 1 gene expression significantly compared with the placebo MI group (P < 0.01 for both). Values for the losartan and enalapril treated MI groups were not statistically different to shams.

We next analysed the expression of the AT$_{1a}$ receptor gene within the myocardium remote from the infarct zone (Figure 5A). The placebo infarct group was not different to the shams, although a trend for increased AT$_{1a}$ receptor gene expression was noted (P = 0.1). Treatment with either losartan or enalapril reduced AT$_{1a}$ receptor gene expression compared with the placebo MI group (P = not significant), and a significantly greater level of AT$_{1a}$ receptor gene expression than in the shams (P < 0.05). The level of AT$_{1b}$ receptor gene (Figure 5B) expression was barely detectable and markedly lower than that observed for the AT$_{1a}$ receptor gene (approx. 1% of the total expression for AT$_{1a}$). Expression of the gene encoding the AT$_{1b}$ receptor was quite similar among the sham and all three MI groups.

DISCUSSION

We previously developed a model of MI in mice to investigate the molecular mechanisms underlying the pathophysiology of LV remodelling following MI [17]. As in our previous series of mice 6 weeks following MI [17], untreated MI mice in the present study demonstrated increases in LV mass and chamber size (determined both by echocardiography and post-mortem, passive pressure-volume relations). In addition, the untreated infarct group exhibited greater collagen content within the non-infarct zone, along with increases in both ANP and collagen type I gene expression. We also noted a modest increase in AT$_{1a}$ receptor gene expression, but no increase in the expression of the AT$_{1b}$ receptor gene.
These results are similar to alterations in myocardial gene expression previously reported in the rat following MI [13,22–24].

In the present study, we further characterized the mouse MI model by evaluating the effects of AT₁ receptor antagonism versus ACE inhibition. Although both agents have been shown to ameliorate ventricular remodelling in the rat following MI, there have been no studies to date to evaluate the effect of these agents on the morphometric and gene expression changes characteristic of post-MI ventricular remodelling in mice. The doses of losartan and enalapril utilized in the present study were carefully chosen based on a preliminary study evaluating hypotensive effects and dose–response relationships. ACE inhibition and AT₁ receptor blockade equally inhibited LV hypertrophy following MI. Indeed, in both drug-treated groups, the LV mass/BM ratio was lower than even the sham group. This finding is not unexpected given that LV mass following MI is determined by the amount of remaining myocardium and the degree of myocardial hypertrophy (the thin apical scar contributes relatively little to overall LV mass). Neither agent, however, mitigated the extent of LV dilatation 6 weeks post-MI in the mouse MI model, an observation that is in clear distinction from the rat MI model [1]. This finding may be due in part to the improved survival of mice with large infarcts in the drug-treated groups, which might mask a modest treatment effect in reducing LV dilatation. Alternatively, infarcts in mice often result in the formation of large apical aneurysms; thus, infarct expansion and stretching may be the major determinant of increased LV volume in this model and may be relatively uninfluenced by enalapril or losartan. Given the small size of the mouse heart, however, it is also possible that methods used to measure murine LV chamber size (passive pressure–volume relations and echocardiography) may not be sensitive enough to detect modest effects of drug treatment.

Within the ventricular myocardium, ANP gene expression is a fetal gene that is re-expressed in cardiac myocytes in response to increased load or injury [20]. In the present study, we observed a strong and significant correlation between ANP gene expression and LV mass/BM. This observation suggests that the increase in LV mass following MI in this model is primarily mediated by cardiomyocyte hypertrophy. Here, ACE inhibition and AT₁ receptor antagonism decreased the level of ANP gene expression, corresponding well to the LV mass data. Taken together, these results suggest that both agents equally limit the extent of cardiomyocyte growth in the mouse heart post-MI.

The untreated MI group exhibited an increase in collagen content within the non-infarct zone that was reduced in both the ACE inhibitor and AT₁ receptor antagonist treated groups. Both treated groups also demonstrated a reduction in collagen type I mRNA, the expression of which is specific to cardiac fibroblasts within myocardium [21]. Thus, ACE inhibition and AT₁ receptor blockade exert similar inhibitory effects on cardiac fibroblasts in the mouse myocardium following MI. Interestingly, neither agent had a significant effect on the expression of the AT₁a receptor gene. Prior experiments in vitro have demonstrated that stretching neonatal rat cardiomyocytes (simulating increased load) increases AT₁a receptor gene expression and receptor number on the cell surface [25,26]. In contrast, stimulation of cardiomyocytes with Ang II results in decreased AT₁a receptor gene expression [25,27]. Our results suggest that the net level of myocardial AT₁a receptor gene expression in the untreated infarct mice may result from a combination of influences. Alterations in loading conditions may be the primary determinant, whereas changes in local neurohormonal levels may play a lesser role in this model. The further increase in AT₁a receptor gene expression in the treated infarct groups may result from positive feedback due to diminished AT₁ receptor activation.

ACE inhibition reduces the extent of cardiac remodelling in many animal models [12–15,28]. The mechanism of benefit of ACE inhibitors is complex, and probably secondary to the combined effects of reduced Ang II formation (both systemically and locally) along with increased local bradykinin levels [29–32]. Many investigators utilizing the rat MI model have demonstrated that AT₁ receptor blockade prevents LV hypertrophy and increased interstitial collagen content [13,31,33]. Blockade of the AT₁ receptor inhibits its activation by Ang II, and leads to the unopposed stimulation of the AT₂ receptor subtype, an effect that may account for some of the observed benefits of AT₁ receptor blockade in the rat heart following MI [31,32]. In contrast with ACE inhibitors, however, AT₁ receptor antagonists do not reduce LV hypertrophy or chamber dilation in some animal models. For example, in the dog model of direct myocardial injury [14] and pig model of chronic tachycardia/dilated cardiomyopathy [15], AT₁ receptor blockade has no effect in reducing LV hypertrophy or dilatation. Nonetheless, studies in rats consistently demonstrate anti-remodelling effects of AT₁ receptor antagonism, particularly following MI, suggesting that significant species variation exists in the myocardial responses to AT₁ receptor blockade. The reason for this is not entirely clear, but may be related to the observed species variations in myocardial levels of Ang II, its receptors and other components important to Ang II signalling [11,16].

Our findings in the mouse MI model are in many respects analogous to those observed in comparative rat MI studies: ACE inhibition and AT₁ receptor blockade equally inhibit myocyte hypertrophy and fibroblast-mediated type I collagen synthesis within the non-infarct zone following MI. Mice are an important species for...
experimental studies in vivo because of the ability to create transgenic strains. The present study therefore serves to establish that in the mouse MI model, ACE inhibition and AT$_1$ receptor antagonism have equivalent inhibitory effects on the gross morphological features and gene expression markers characteristic of post-MI ventricular remodelling.

Summary

In a murine model of chronic MI, we have demonstrated that ACE inhibition and AT$_1$ receptor blockade immediately following MI both reduce myocardial hypertrophy, ANP gene expression, interstitial collagen content and collagen type I gene expression to equivalent degrees. These results serve as an important starting point for planned investigations to evaluate the anti-remodelling effects of these agents on mice in which genetic manipulations are used to disrupt components of the Ang II signalling system.

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19. Reference deleted

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