Survival After Myocardial Infarction in the Rat
Role of Tissue Angiotensin-Converting Enzyme Inhibition

Kai C. Wollert, MD; Roland Studer, PhD; Bodil von Bülow, BS; Helmut Drexler, MD

Background Chronic treatment with high doses of angiotensin-converting enzyme (ACE) inhibitors prolongs survival after myocardial infarction. Since the plasma renin-angiotensin system (RAS) is not consistently activated in the chronic phase after myocardial infarction, the beneficial effects of ACE inhibition have been attributed, in part, to inhibition of an activated tissue RAS. However, a relation between tissue ACE inhibition and long-term efficacy (ie, concerning left ventricular [LV] hypertrophy and survival) has not been established. The present study was designed to evaluate the impact of low-dose ACE inhibition (predominant inhibition of plasma ACE) and high-dose ACE inhibition (associated with substantial tissue ACE inhibition) on reversal of LV hypertrophy and 1-year mortality after myocardial infarction in the rat.

Methods and Results Infarcted rats were randomized to placebo, low-dose lisinopril, or high-dose lisinopril (each, n = 80) and compared with sham-operated animals (n = 40). In a separate group of animals, tissue ACE activity was determined after 6 weeks of therapy, demonstrating that both regimens were effective with regard to both plasma and pulmonary ACE inhibition; however, only high-dose lisinopril inhibited renal ACE. Neither dose affected LV ACE activity and ACE mRNA levels as determined by competitive polymerase chain reaction, whereas LV ANF mRNA levels were significantly reduced by high-dose lisinopril. High-dose lisinopril reduced arterial blood pressure and normalized right ventricular and LV weight and resulted in a substantial reduction of 1-year mortality, whereas the low dose did not (1 year mortality: placebo, 56.3%; low dose, 53.3%; high dose, 22.9%, P < .001 versus low dose and versus placebo).

Conclusions Hemodynamically effective ACE inhibition is required for reduction of LV hypertrophy and long-term mortality after myocardial infarction in the rat. Sustained inhibition of renal ACE during long-term therapy may contribute to the beneficial effect of high-dose lisinopril. Low-dose lisinopril, although exerting sustained inhibition of the plasma ACE, does not improve survival after myocardial infarction. (Circulation. 1994;90:2457-2467.)

Key Words • hypertrophy • angiotensin enzymes RNA • lisinopril • polymerase chain reaction

The rat model of chronic myocardial infarction (MI) induced by coronary artery ligation represents a well-characterized animal model of chronic congestive heart failure. MI in the rat model results in a profound alteration of ventricular architecture characterized by early expansion of the infarcted area,1 ventricular dilatation,2 and hypertrophy of the noninfarcted left ventricle (LV).3 This cardiac remodeling process can be attenuated by long-term treatment with the hemodynamically effective angiotensin-converting enzyme (ACE) inhibitor captopril,4 resulting in improved long-term survival.5 On the basis of this beneficial effect of captopril in the rat infarct model, clinical trials have now provided convincing evidence that this therapeutic principle also applies for patients with large MI.5 Since the plasma renin-angiotensin system (RAS) is not consistently activated after MI,7,8 the beneficial effects of ACE inhibition have been attributed, in part, to inhibition of an activated tissue RAS. Indeed, recent studies strongly suggest a selective activation of the tissue ACE in the heart and in the kidneys after MI in the rat, which could represent a target for ACE inhibitors.9,10 Evidence is accumulating that the cardiac RAS is involved in the stretch-mediated hypertrophic response of cardiac myocytes.11-13 Conceivably, interfering with the activated cardiac RAS may prevent the development of LV remodeling and hypertrophy after MI. However, data demonstrating a relation between the degree of cardiac tissue ACE inhibition and the effect of ACE inhibitors on LV hypertrophy and survival are lacking. Alternatively, the beneficial effects of long-term ACE inhibition on survival after MI may be predominantly due to systemic effects and inhibition of renal tissue ACE, thereby reducing preload and afterload.

In the clinical setting, ACE inhibitors are often prescribed in very low doses, assuming that low doses may be as effective and have fewer side effects compared with the recommended dose.14 Indeed, experimental data obtained from a high renin–pressure overload model suggested that ACE inhibitors can prevent LV hypertrophy in nonhypotensive doses.15 To the contrary, most clinical trials have used high target doses of ACE inhibitors for the treatment of chronic heart failure.6,16,17 Accordingly, the present study was designed to evaluate the impact of low-dose ACE inhibition (predominant inhibition of plasma RAS) and high-dose ACE inhibition (associated with substantial tissue ACE inhibition) on reversal of LV hypertrophy and 1-year mortality after MI in the rat.

Methods

Experimental Infarction Male Sprague-Dawley rats weighing 250 to 300 g were obtained from the Zentralinstitut für Versuchstierkunde, Hannover, FRG, and housed in our animal facility for at least 4
days before operation. The animal studies were approved by the Ethical Committee for Animal Research at the University of Freiburg, FRG. Coronary artery ligation was performed as previously described.18 Brieﬂy, rats were anesthetized with pentobarbital and ketamine. The left coronary artery was ligated approximately 2 mm distal to its aortic origin with a 6.0 Prolene suture. There was an ~40% mortality rate within the first 48 hours. In sham-operated animals, the suture was tied loosely so as not to obstruct coronary ﬂow. Animals were allowed free access to a 0.5% sodium standard rat chow. Drinking water was provided ad libitum.

Administration of Lisinopril

The ACE inhibitor lisinopril was dissolved in the drinking water at concentrations of 3.85 mg/L (low dose) and 38.5 mg/L (high dose). The dose selection was based on a pilot study demonstrating that these dosages result in serum lisinopril levels comparable to a low dose of approximately 2.5 to 5 mg/d or a high dose of approximately 20 mg/d.21 The high dose was equivalent to the target dose in large clinical trials.14,15 The pilot study also documented an effective inhibition of plasma ACE activity and a signiﬁcant rightward shift of the angiotensin (Ang) I pressure-response curve during both low- and high-dose treatment.

Instrumentation

Rats were anesthetized with halothane (1% in oxygen). Saline-ﬁlled catheters (PE 50) were inserted into the right carotid artery and jugular vein. The arterial line was connected to a Statham P23ID pressure transducer. Blood pressure tracings were displayed on a recorder screen (Elema Mingograph, Siemens). After closure, rats were allowed to recover for a minimum of 3 hours before hemodynamic measurements were performed. This recovery period is of sufﬁcient duration to ensure a return to baseline conditions in the rat.22

Angiotensin Challenges

To document effective and sustained plasma ACE inhibition (particularly during low-dose lisinopril therapy), Ang I and Ang II dose-response curves were obtained at 2 weeks and 1 year of therapy. Baseline arterial blood pressure and heart rate were recorded. Subsequently, pressure responses to Ang I and Ang II (Sigma) were determined. Increasing doses of Ang I and Ang II (1, 3, 10, 30, 100, 300, and 1000 nM) were injected intravenously in 0.1 mL saline. A 5-minute interval was maintained between pressure stimuli. The angiotensin dosages were increased until a 20 mm Hg peak change in mean arterial blood pressure was observed. Dose-response curves for Ang I and Ang II were derived from the peak changes in mean arterial pressure.

Determination of Infarct Size

The LV was ﬁxed in 10% formalin and embedded in parafﬁn. Six transverse slices were cut from apex to base. A 5-μm section was cut from each slice and mounted. The sections were stained with sirius red (0.1% solution in saturated aqueous picric acid) to provide a clear discrimination between ﬁbrous scar and noninfarcted tissue.23 Infarct size was determined by planimetric measurement with a digital image analyzer (Leitz). To calculate infarct size, the ratios of scar length to circumference from each of the six slices were averaged and expressed as percentage.

Tissue Sampling

Rats were killed in the early morning by decapitation. Trunk blood was collected into chilled tubes containing either heparin (200 IU/mL) or EDTA disodium salt (1 mg/mL). The tubes were immediately centrifuged at 4°C. Plasma containing heparin was snap-frozen and stored in liquid nitrogen until assay of ACE activity. Plasma containing EDTA was divided into two samples. The ﬁrst sample was stored at ~80°C for later determination of plasma renin activity. Aprotinin (500 KIU/mL plasma, Bayer) was added to the second sample, which was then stored in liquid nitrogen for later assay of atrial natriuretic peptide (ANF). The thoracic contents were removed en bloc. Major pulmonary trunks were removed from the lungs. The atria and the right ventricle were dissected from the LV. The infarct border and the border zone were separated from viable LV myocardium. In sham-operated animals, corresponding parts of the LV were discarded. Only viable LV myocardium (representing the inferior wall and the interventricular septum) was used for the subsequent analyses. The kidneys were freed of their capsules. All tissues were rinsed in ice-cold saline, blotted dry, weighed, and snap-frozen. Tissues were either stored in liquid nitrogen for subsequent assay of ACE activity or stored at ~80°C for later isolation of RNA.

Biochemical Measurements

Serum lisinopril concentration was determined by radioimmunoassay.24 Plasma renin activity was measured by radioimmunoassay of Ang I generated by incubation with an excess of rat renin substrate.25 After extraction, plasma ANF concentrations were determined by radioimmunoassay as previously described.26 Plasma ACE activity for the ACE inhibitor was determined by the rate of generation of [14C]- hippuric acid from [14C]His-Leu (HHL) substrate using the optimal incubation conditions for cleavage of HHL.27 Tissues were homogenized in ice-cold 0.3% Triton X-100 by use of a 12-second impulse from an Ultra Turrax homogenizer. After centrifugation (20 000g, 20 minutes, 4°C), 50 μL of the supernatant or 50 μL of plasma was incubated with 150 μL of assay buffer at 37°C in a water bath. The assay buffer consisted of 300 mmol/L NaCl, 100 mmol/L NaHPO4 (pH 8.5), 5 mmol/L HHL (Sigma), and 0.1 mmol/L [14C]HHL (speciﬁc activity, 3 mCi/mmol; Amersham). The [14C] hippuric acid generated was extracted with ethylacetate and quanitﬁed in a scintillation counter. Tissue ACE activity was expressed as nmoles HHL turnover · mg⁻¹ · min⁻¹ tissue protein. The protein content of the tissue supernatant was determined by the method of Bradford. Plasma ACE activity was expressed as nmoles HHL turnover · mL⁻¹ · min⁻¹ plasma.

Preparation and Quantification of RNA

Frozen LV tissue samples were disintegrated with a Mikro Dismembrator (B. Braun). Total cellular RNA was isolated by the acid guanidinium thiocyanate/phenol/chloroform extraction method as described by Chomczynski and Sacchi.28 The RNA was dissolved in diethylpyrocarboxonate-treated water and quantiﬁed by absorbance at 260 nm in triplicate. Aliquots were subjected to agarose gel electrophoresis and stained with ethidium bromide to conﬁrm absence of RNA degradation by demonstrating the integrity of the ribosomal 18s and 28s bands.

Northern Blot Analysis

Total RNA was subjected to formaldehyde agarose gel electrophoresis29 and transferred to a nylon membrane by overnight capillary blotting. The filters were successively hybridized with a 0.6-kb rat ANF cDNA probe (Pat/PS1)30 and a 1.2-kb chicken β-actin cDNA probe (EcoRI/HindIII).31 Before cDNA hybridization, the filters were preincubated for 4 hours at 65°C in hybridization buffer containing 50 μg/mL sonicated and denaturated Escherichia coli DNA (Sigma), 2× standard saline citrate (SSC; 1× SSC is 15 mmol/L sodium citrate and 0.15 mol/L NaCl), 10× Denhardt’s solution, 0.1% SDS, 0.1% sodium pyrophosphate, 2 mmol/L EDTA, and 2.5% dextran sulfate. The cDNA probes were labeled with [α35S]PdCTP by random priming (Multiprime DNA Labeling Kit, Amersham). Approximately 1 × 106 cpm of the labeled cDNA probe was added to 10 mL of hybridization buffer, and the filters were hybridized overnight at 65°C. Subsequently, the filters were washed under appropriate conditions of stringency (2× SSC/0.1% SDS, 0.5× SSC/0.1% SDS, 0.1× SSC/0.1% SDS).
SDS, 30 minutes each, at 65°C). The filters were exposed to x-ray films (X-OMAT AR, Kodak), and the autoradiograms were scanned with a laser densitometer (Personal Densitometer, Molecular Dynamics). The extent of ANF cDNA hybridization was normalized to the hybridization signals obtained with the β-actin cDNA to correct for differences in loading and/or transfer efficiencies.

**Competitive Reverse Transcription–Polymerase Chain Reaction**

The expression of ACE mRNA in the noninfarcted LV was measured by competitive reverse transcription–polymerase chain reaction (RT-PCR). In competitive RT-PCR, the template mRNA and definite numbers of competitor RNA molecules are first reverse transcribed and subsequently amplified in the same reaction tube.32,33 In a deletion within the competitor RNA molecules allows the PCR products of the competitor and the template to be separated by gel electrophoresis. The PCR products generated by the competitor and the template are then quantified to calculate the initial amount of template mRNA molecules.

**Construction of the Competitor RNA**

To obtain an internal control competitor RNA, a shortened fragment of the human ACE cDNA was constructed and transcribed into cRNA. The human ACE cDNA was sequenced to a PCR with the sense primer 5'-CGCTACAACTTCGACTG-TCGACTTGTTG-3' and the antisense primer 5'-GGG-TAAACCGAGGATG-3'. The PCR yielded a 951-bp (1481 to 2431) cDNA fragment. The cDNA fragment was phosphorylated, blunted, ligated into a blunt-ended, dephosphorylated Bluescript SK(-) vector (Stratagene), and amplified on GM 2929 (dem) E. coli bacterial strain. A 315-bp fragment was removed by digestion with the restriction enzyme PstMI. The shortened cDNA fragment was separated on a low-melting-point agarose gel, blunted, and religated. The shortened cDNA clone was linearized with EcoRI. Subsequently, 1 µg was transcribed into cRNA with a T3-/T7-RNA polymerase in vitro transcription kit (Amersham). The cDNA template was then removed by addition of 15 U RNase-free DNase (Life Technologies) and incubation at 37°C for 30 minutes. The competitor cRNA was recovered by phenol extraction and ethanol precipitation and quantified by absorption at 260 nm. The cRNA solution was stabilized by addition of 30 µg/mL of yeast tRNA (Life Technologies) and stored at -20°C. No remaining cDNA could be detected when the competitor cRNA was subjected to RT-PCR with reverse transcriptase omitting in the first step.

**Selection and Synthesis of the PCR Primers**

The cDNA sequence of the rat ACE has not been published. We therefore selected sense and antisense oligonucleotide primers from the human ACE cDNA sequence35 that showed a complete sequence homology to the murine ACE cDNA36,37: sense primer 1481 to 1501 5'-CGCTACAACTTCGACTG-TCGACTTGTTG-3', antisense primer 2342 to 2361 5'-TATTITCCGG-GATGTCGCAT-3'. The primers were selected by computer analysis (Oligo program, National Biosciences) and were synthesized by use of a DNA synthesizer (Applied Biosystems). To ensure that no genomic DNA contamination was present in the LV RNA solutions subjected to RT-PCR, the oligonucleotide primers spanned several splice junctions.38

**Reverse Transcription**

For first-strand cDNA synthesis, a set of reactions was prepared by addition of increasing quantities of competitor cRNA molecules (0.25 × 10^6 to 6.0 × 10^7) to a constant amount of total LV RNA (1.25 µg). Reactions were carried out in a final volume of 25 µL containing 1× reverse transcription buffer (50 mmol/L Tris-HCl, pH 8.3, 75 mmol/L KCl, 3 mmol/L MgCl₂, 0.5 mmol/L dNTPs (Pharmacia), and 250 pmol of random hexanucleotide primers. The mixtures were heated at 72°C for 3 minutes followed by cooling to 42°C. Subsequently, dithiothreitol (final concentration, 10 mmol/L), RNase inhibitor (2 U/100 ng of total RNA, Life Technologies), and Moloney murine leukemia virus reverse transcriptase (10 U/100 ng of total RNA, Life Technologies) were added, and the reaction mixtures were incubated at 42°C for 60 minutes. As a negative control, one additional reaction mixture was set up omitting the reverse transcriptase.

**PCR Amplification**

Duplicate samples of PCR reaction were performed in a total volume of 50 µL, respectively, each containing 10 µL of reverse transcription reaction, 35 µL of a PCR master mix (20 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 0.5 mmol/L MgCl₂, 20 pmol sense and antisense primer), and 2.5 U Thermus aquaticus DNA polymerase (Amersham Buchler Ltd). Twenty-eight PCR amplification cycles were run with the following cycle profile: denaturation (1 minute at 94°C), annealing (2 minutes at 65°C), and extension (3 minutes at 72°C).

**Quantitative Analysis**

See Fig 1. Ten microliters from each PCR reaction was loaded onto 1.5% agarose gels containing 0.5 µg/mL ethidium bromide. After electrophoresis, the amplification products of the template and the competitor were visualized by UV illumination, and a photograph was taken. The negative films were used to evaluate the band intensities by laser densitometry (Personal Densitometer). To correct for the difference in molecular weight, the band densities of the ACE competitor product were multiplied by 1.56 (881 bp divided by 566 bp). The ratios of the corrected band densities of the competitor products to the band densities of the template products were calculated, and the logarithmic values were plotted against the logarithm of the known number of competitor cRNA molecules. At the point at which the products of the competitor cRNA and the template RNA are in equivalence (log ratio=0), the starting concentration of the template RNA equals the starting concentration of the competitor RNA. The linearity of the competitive RT-PCR was verified by subjecting five dilutions of LV RNA (0, 0.313, 0.625, 1.25, and 1.875 µg) to reverse transcription and subsequent PCR amplification. This resulted in a linear relationship (r=0.99, P<.001). The competitive RT-PCR was reproducible with repetitive measurements from individual samples differing <15% from each other.

**Protocol 1**

Six to 8 days after coronary artery ligation, rats were randomly assigned to vehicle (MI-V) or low-dose (MI-L) or high-dose lisinopril (MI-H, n=7 to 10 per group). After treatment for 14 days, catheters were inserted into the right carotid artery and jugular vein. A 0.4-mL venous blood sample was withdrawn for determination of serum lisinopril concentration. After recovery (>3 hours), baseline arterial blood pressure and heart rate were measured in resting conscious rats. Subsequently, blood pressure response curves to intravenous Ang I and Ang II were recorded. At the end of the protocol, the hearts were removed after halothane anesthesia and placed in 10% formalin for later determination of infarct size. The same protocol was carried out after treatment for 1 year using animals that completed protocol 3 (n=6 to 8 per group).

**Protocol 2**

Six to 8 days after coronary artery ligation, rats were randomized to receive vehicle (MI-V, n=16) or low-dose (MI-L, n=17) or high-dose lisinopril (MI-H, n=14). Sham-operated animals were treated with vehicle (SH-V, n=16). After treatment for 6 weeks, rats were killed by decapitation.
and trunk blood was collected for measurement of plasma ACE activity. The noninfarcted parts of the LV, the lungs, and the kidneys were used for determination of tissue ACE activities. In a subset of animals, we used one half of the noninfarcted LV for isolation of total RNA and measurement of ACE mRNA and ANF mRNA levels (MI-V, MI-L, MI-H, n=9 per group; SH-V, n=12). In addition, plasma renin activities and plasma ANF concentrations were determined in these animals. Since LV tissue was used for biochemical analyses, a crude estimate of transverse length of the infarct size was determined from the fixed transverse sections sampled at the middle (equatorial) part of each LV. Additional groups of animals (n=4 per group) were treated as outlined above for 1 day only, then killed, and cardiac tissue ACE activities were measured.

Protocol 3

To assess the influence of low-dose and high-dose lisinopril on 1-year mortality, 378 rats underwent coronary artery ligation. One hundred thirty-eight rats died within the first 3 days (mortality, 36.5%). Six to 8 days after the operative procedure, the surviving 240 animals were randomly assigned to vehicle (MI-V) or low-dose (MI-L) or high-dose lisinopril (MI-H, n=80 per group). A sham operation was performed in 40 rats, and these animals were treated with vehicle (SH-V). All animals were followed until death or for up to 1 year after surgery. Cages were inspected daily for dead animals. The date of each death and the weight of the dead animal were recorded. The lungs, the atria, the right ventricle, and the LV were weighed, and the LV was placed in 10% formalin for planimetric determination of infarct size. One year after surgery, all surviving animals were weighed and anesthetized with halothane. The thoracic contents were removed, and again, the organ weights were recorded and the LVs were collected for measurement of infarct size. According to a classification used by Pfeffer et al,3 infarcts were classified as small (<20%), moderate (20 to <40%), and large (>40%).

Statistical Analyses

Data are presented as mean±SEM. Intergroup variables were first compared by one-way ANOVA. Four prespecified comparisons were subsequently made by Bonferroni's t test (MI-V versus SH-V, MI-L versus MI-V, MI-H versus MI-V, and MI-H versus MI-L). Linear regression analysis was performed to test for a correlation between two variables. Survival data are presented as Kaplan-Meier curves. The survival curves of individual groups were compared by the log-rank test. To account for multiple testing in pairwise comparisons, a closed test procedure was used.39 In addition, the mortality data from protocol 3 were stratified according to infarct size and analyzed by a Cox proportional-hazard model. A two-tailed value of P<.05 was considered to indicate significance.

Results

Protocol 1

The effects of low-dose and high-dose lisinopril on resting arterial blood pressure, heart rate, and pressure responses to Ang I and Ang II were determined in two separate sets of animals after treatment for 2 weeks or for 1 year, respectively (Table 1, Fig 2). There were no significant differences in infarct size between the treatment groups. Plasma lisinopril concentrations were significantly higher in high-dose compared with low-dose treatment (P<.05, Table 1). With low-dose lisinopril, a
significant decrease in systolic \((P<.01)\) and diastolic \((P<.05)\) blood pressures was observed only at 2 weeks (Table 1). High-dose lisinopril caused a significant decrease of systolic and diastolic blood pressure at 2 weeks \((P<.001)\) and at 1 year \((P<.01)\) (Table 1). Reduction of blood pressure with high-dose lisinopril was significantly stronger compared with low-dose treatment \((P<.05)\). There were no significant changes in heart rate during lisinopril treatment. The pressure-response curves to Ang I and Ang II are shown in Fig 2a through 2d. Both low- and high-dose lisinopril caused a significant, dose-dependent rightward shift of the Ang I pressure-response curve, whereas the pressure response to Ang II was well preserved. The pressure-response

Table 1. Effects of Low-Dose and High-Dose Lisinopril on Blood Pressure, Heart Rate, and Serum Lisinopril Concentration

<table>
<thead>
<tr>
<th></th>
<th>Systolic BP, mm Hg</th>
<th>Diastolic BP, mm Hg</th>
<th>HR, bpm</th>
<th>Lis, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2-Week treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI-V (n=8)</td>
<td>26±4</td>
<td>142±6</td>
<td>110±5</td>
<td>410±15</td>
</tr>
<tr>
<td>MI-L (n=10)</td>
<td>26±3</td>
<td>122±2†</td>
<td>92±2*</td>
<td>360±18</td>
</tr>
<tr>
<td>MI-H (n=7)</td>
<td>26±4</td>
<td>107±3§</td>
<td>76±5‡§</td>
<td>377±12</td>
</tr>
<tr>
<td><strong>1-Year treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI-V (n=6)</td>
<td>22±2</td>
<td>134±5</td>
<td>100±4</td>
<td>357±18</td>
</tr>
<tr>
<td>MI-L (n=6)</td>
<td>19±2</td>
<td>129±4</td>
<td>96±5</td>
<td>350±28</td>
</tr>
<tr>
<td>MI-H (n=8)</td>
<td>23±4</td>
<td>111±3§</td>
<td>80±3‡§</td>
<td>326±12</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Mi indicates infarct size; BP, arterial blood pressure; HR, heart rate; bpm, beats per minute; Lis, serum lisinopril concentration; MI-V, MI-L, MI-H, infarcted rats treated with vehicle or low-dose or high-dose lisinopril, respectively; and n.d., not determined. Treatment was initiated 6 to 8 days after surgery and lasted for either 2 weeks or 1 year. \(*P<.05, \dagger P<.01, \ddagger P<.001\) vs MI-V; \(§P<.05\) vs MI-L.

Fig 2. Graphs showing effects of low-dose and high-dose lisinopril on pressure responses to angiotensin (Ang) I and II. Values are mean±SEM. MI-V, MI-L, and MI-H indicate infarcted rats treated with vehicle or low-dose or high-dose lisinopril, respectively. Same animals as in Table 1. Angiotensin I and II challenges were performed in two different sets of animals after treatment for 2 weeks or for 1 year. Both low and high doses caused a significant rightward shift \((P<.05\) for low dose vs placebo and \(P<.01\) for high dose vs placebo). MAP indicates mean arterial pressure.
curves were similar after treatment for 2 weeks and for 1 year. The rightward shift of the Ang I dose-response curve was more pronounced with high-dose lisinopril.

Protocol 2

To assess the impact of chronic lisinopril treatment on ventricular weights and plasma and tissue ACE activities, infarcted rats were treated with vehicle (MI-V, n=16) or low-dose (MI-L, n=17) or high-dose (MI-H, n=14) lisinopril for 6 weeks. Sham-operated animals received vehicle only and served as a control group (SH-V, n=16). A treatment period of 6 weeks was chosen because the mortality rate of placebo-treated rats increases substantially beyond 2 months after MI. Therefore, an investigation at a later stage would have resulted in a selection in favor of treated animals. To confirm an equal distribution of infarct sizes among the three infarcted groups, a crude estimate of transversal length of the infarct size was obtained from fixed transverse sections sampled at the equatorial part of the LV. These estimates of infarct size did not differ significantly between the three groups (Table 2). Body weights did not differ between the experimental groups. There were no significant differences in ventricular weights in vehicle-treated infarcted rats compared with sham-operated rats. Compared with vehicle treatment, only high-dose lisinopril caused a significant decrease in the absolute and relative weights of the LV (P<.01).

Table 2. Effects of Low-Dose and High-Dose Lisinopril on Plasma and Tissue ACE Activities

<table>
<thead>
<tr>
<th></th>
<th>BW, g</th>
<th>Infarct Size, %</th>
<th>Plasma, nmol · min⁻¹ · mL⁻¹</th>
<th>Lungs</th>
<th>Kidney, nmol · min⁻¹ · mg prot⁻¹</th>
<th>nLV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH-V (n=16)</td>
<td>475±8</td>
<td>0</td>
<td>48±7</td>
<td>179±19</td>
<td>0.65±0.07</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>MI-V (n=16)</td>
<td>492±15</td>
<td>43±4</td>
<td>57±5</td>
<td>128±18</td>
<td>0.82±0.14</td>
<td>0.45±0.04</td>
</tr>
<tr>
<td>MI-L (n=17)</td>
<td>461±10</td>
<td>39±2</td>
<td>27±2*</td>
<td>45±6*</td>
<td>0.63±0.08</td>
<td>0.45±0.06</td>
</tr>
<tr>
<td>MI-H (n=14)</td>
<td>471±14</td>
<td>42±4</td>
<td>13±2*</td>
<td>26±5*</td>
<td>0.21±0.04</td>
<td>0.43±0.04</td>
</tr>
</tbody>
</table>

ACE activity was significantly decreased by lisinopril treatment. Plasma renin activity in vehicle-treated infarcted and sham-operated rats did not differ significantly. An increase in plasma renin activity was observed during low-dose and high-dose lisinopril treatment (P<.001, Table 3).

By use of competitive RT-PCR, ACE mRNA levels were measured in the noninfarcted parts of the LV. As shown in Fig 3, the number of ACE transcripts per 500 ng total RNA significantly increased in vehicle-treated infarcted rats compared with sham-operated rats (4.7±1.0*10⁴ versus 2.6±1.0*10⁴, P<.001). Infarcted animals treated with low doses or high doses of lisinopril did not differ from vehicle-treated infarcted animals with regard to LV ACE mRNA expression. There was a significant positive correlation between LV ACE mRNA expression and ACE activity (n=21, r=0.75, P=.007, Fig 4).

A 25-fold increase in LV ANF mRNA expression was observed in infarcted animals treated with vehicle (P<.001). Only treatment with high-dose lisinopril caused a significant reduction in ANF mRNA expression (P<.05). Similar results were obtained when ANF mRNA was normalized to β-actin (Fig 5). The expression of β-actin did not differ significantly between the four experimental groups (data not shown).

In separate groups of animals treated for only 1 day, cardiac ACE activity was increased in placebo-treated infarcted rats compared with sham-operated animals. Both low- and high-dose lisinopril inhibited cardiac ACE activity (SH-V/MI-V/MI-L/MI-H, 0.44±0.07/0.78±0.19/0.18±0.03/0.11±0.07 nmol · min⁻¹ · mg protein⁻¹).

Table 3. Effects of Low-Dose and High-Dose Lisinopril on Plasma Renin Activity and Atrial Natriuretic Peptide Concentration

<table>
<thead>
<tr>
<th></th>
<th>MI Size, %</th>
<th>PRA, ng · mL⁻¹ · min⁻¹</th>
<th>ANF, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH-V (n=12)</td>
<td>0</td>
<td>4.6±1.0</td>
<td>128±25</td>
</tr>
<tr>
<td>MI-V (n=9)</td>
<td>43±3</td>
<td>6.4±1.3</td>
<td>505±106*</td>
</tr>
<tr>
<td>MI-L (n=9)</td>
<td>39±3</td>
<td>23.4±1.3*</td>
<td>424±85</td>
</tr>
<tr>
<td>MI-H (n=9)</td>
<td>39±3</td>
<td>20.2±1.7*</td>
<td>316±69</td>
</tr>
</tbody>
</table>

MI size indicates infarct size determined in the equatorial plane of the left ventricle; PRA, plasma renin activity; ANF, atrial natriuretic peptide; SH-V, sham-operated rats treated with vehicle; and MI-V, MI-L, MI-H, infarcted rats treated with vehicle or low-dose or high-dose lisinopril, respectively. Treatment was started 6 to 8 days after surgery and lasted for 6 weeks. Same animals as in Figs 2, 3, and 4. Values are mean±SEM. *P=.01 vs SH-V; †P<.001 vs MI-V.
transcriptase-polymerase enzyme.

...tein 

Fig 3. Bar graph showing ACE mRNA expression in the noninfarcted parts of the left ventricle. Values are mean±SEM. SH-V indicates sham-operated rats treated with vehicle (n=12); MI-V, MI-L, and MI-H, infarcted rats treated with vehicle or low-dose or high-dose lisinopril, respectively (n=9 in each group). Treatment was initiated 6 to 8 days after surgery and lasted for 6 weeks. ACE mRNA expression was quantified by competitive reverse transcription–polymerase chain reaction. ACE indicates angiotensin-converting enzyme.

P<.05 for SH-V versus MI-V and P<.01 for MI-L and MI-H versus MI-V).

Protocol 3

The survival curves of the four experimental groups are depicted in Fig 6. None of the sham-operated rats died during the 1-year observation period. Vehicle-treated infarcted animals exhibited a 1-year mortality rate of 56.3% (P<.00001 MI-V versus SH-V). Treatment with low-dose lisinopril did not result in a reduction of the 1-year mortality rate (MI-L, 53.3%; P=.48 MI-L versus MI-V). However, the 1-year mortality rate was significantly reduced by high-dose lisinopril (MI-H, 22.9%; P<.00001 MI-H versus MI-V). High-dose treatment was superior to low-dose treatment (P<.0001 MI-H versus MI-L). In an additional analysis, the mortality data from the three infarcted groups were stratified according to infarct size and analyzed by a Cox proportional-hazards model. In relation to the MI-V group, the relative risk to die within the 1-year obser-

Fig 4. Graph showing correlation between angiotensin-converting enzyme (ACE) mRNA expression and ACE activity in the noninfarcted parts of the left ventricle. Treatment was initiated 6 to 8 days after surgery and lasted for 6 weeks. Combined analysis from vehicle-treated sham-operated (n=12) and infarcted animals (n=9).

Fig 5. Bar graph showing influence of low-dose and high-dose lisinopril on atrial natriuretic peptide (ANF) mRNA expression (normalized to β-actin mRNA) in the noninfarcted parts of the left ventricle. Values are mean±SEM. SH-V indicates sham-operated rats treated with vehicle (n=12); MI-V, MI-L, and MI-H, infarcted rats treated with vehicle or low-dose or high-dose lisinopril, respectively (n=9 in each group). Treatment was initiated 6 to 8 days after surgery and lasted for 6 weeks.

Fig 6. Bar graph showing influence of low-dose and high-dose lisinopril on 1-year mortality in infarcted rats. SH-V indicates sham-operated rats treated with vehicle (n=40); MI-V, MI-L, and MI-H, infarcted rats treated with vehicle or low-dose or high-dose lisinopril (n=80 per group). Treatment started 6 to 8 days after surgery. Rats were followed until death or for up to 1 year after surgery.
TABLE 4. Effects of Low-Dose and High-Dose Lisinopril on 1-Year Mortality Rates in Rats With Small, Moderate, and Large Infarcts

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>MI, %</th>
<th>1-Year Mortality Rate, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small infarcts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI-V</td>
<td>31</td>
<td>13±1</td>
<td>38.7</td>
<td></td>
</tr>
<tr>
<td>MI-L</td>
<td>26</td>
<td>14±1</td>
<td>35.4</td>
<td>.58 vs MI-V</td>
</tr>
<tr>
<td>MI-H</td>
<td>23</td>
<td>12±1</td>
<td>8.9</td>
<td>&lt;.02 vs MI-V; &lt;.05 vs MI-L</td>
</tr>
<tr>
<td>Moderate infarcts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI-V</td>
<td>45</td>
<td>27±1</td>
<td>64.4</td>
<td></td>
</tr>
<tr>
<td>MI-L</td>
<td>43</td>
<td>29±1</td>
<td>56.6</td>
<td>.26 vs MI-V</td>
</tr>
<tr>
<td>MI-H</td>
<td>50</td>
<td>29±1</td>
<td>22.0</td>
<td>&lt;.0001 vs MI-V; &lt;.001 vs MI-L</td>
</tr>
<tr>
<td>Large infarcts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MI-V</td>
<td>4</td>
<td>43±2</td>
<td>100.0</td>
<td>NS for all comparisons</td>
</tr>
<tr>
<td>MI-L</td>
<td>11</td>
<td>43±1</td>
<td>81.8</td>
<td></td>
</tr>
<tr>
<td>MI-H</td>
<td>7</td>
<td>44±2</td>
<td>83.3</td>
<td></td>
</tr>
</tbody>
</table>

MI indicates infarct size, mean±SEM; MI-V, MI-L, MI-H, infarcted rats treated with vehicle or low-dose or high-dose lisinopril, respectively. Treatment started 6 to 8 days after surgery. Rats were followed until death or for up to 1 year. Rats were classified according to infarct size as having small (<20%), moderate (20% to <40%), or large (≥40%) infarcts.

rats. High-dose treatment resulted in a significant reduction of ventricular weights compared with placebo and low-dose treatment. Since more rats of the placebo and low-dose infarct groups died before the end of the 1-year treatment period compared with the high-dose group, the development of higher cardiac weights in the placebo and low-dose groups is likely to underestimate the extent of cardiac hypertrophy that might have emerged if these animals had lived longer.

TABLE 5. Effects of Low-Dose and High-Dose Lisinopril on Absolute and Relative Right and Left Ventricular Weights After 1 Year of Treatment

<table>
<thead>
<tr>
<th></th>
<th>SH-V (n=40)</th>
<th>MI-V (n=80)</th>
<th>MI-L (n=80)</th>
<th>MI-H (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI, %</td>
<td>0</td>
<td>22±1</td>
<td>26±1</td>
<td>25±1</td>
</tr>
<tr>
<td>BW, g</td>
<td>630±8</td>
<td>554±8§</td>
<td>546±6</td>
<td>581±6*#</td>
</tr>
<tr>
<td>RV, g</td>
<td>0.30±0.01</td>
<td>0.47±0.02§</td>
<td>0.44±0.02</td>
<td>0.38±0.01+b</td>
</tr>
<tr>
<td>RV/BW, g/kg</td>
<td>0.48±0.01</td>
<td>0.87±0.04§</td>
<td>0.83±0.04</td>
<td>0.66±0.03‡*</td>
</tr>
<tr>
<td>LV, g</td>
<td>1.30±0.02</td>
<td>1.35±0.02</td>
<td>1.28±0.02</td>
<td>1.14±0.02‡*</td>
</tr>
<tr>
<td>LV/BW, g/kg</td>
<td>2.08±0.04</td>
<td>2.48±0.04§</td>
<td>2.36±0.04</td>
<td>1.98±0.03‡*</td>
</tr>
</tbody>
</table>

SH-V indicates sham-operated rats treated with vehicle; MI-V, MI-L, MI-H, infarcted rats treated with vehicle or low-dose or high-dose lisinopril, respectively; MI, infarct size in percent of the left ventricle; BW, body weight; RV and LV, absolute right and left ventricular weights; and RV/BW and LV/BW, relative right and left ventricular weights. Values are mean±SEM. Treatment was initiated 6 to 8 days after surgery. Rats were followed until death or up to 1 year.

Discussion

The present study provides the first evidence that the effect of long-term ACE inhibition on survival after MI is dose-dependent. Low doses of an ACE inhibitor, although exerting effective inhibition of the plasma RAS, may not improve survival. In contrast, high doses (equivalent to doses used in clinical trials), associated with renal tissue ACE inhibition, sustained reduction of blood pressure, and prevention of LV hypertrophy, result in a dramatic decrease of long-term mortality in the rat model.

Influence of Low-Dose and High-Dose Lisinopril on Long-term Mortality

The beneficial effects of chronic ACE inhibition on long-term survival after MI were first demonstrated in the rat infarct model. The results of this animal study provided the basis for the design of the subsequent clinical trials, which, in turn, confirmed the experimental findings demonstrating that ACE inhibitors reduce mortality in patients after MI. Thus, this animal model has proved to be extremely useful for studying pharmacological aspects of LV dysfunction after MI. The impressive results of the clinical trials have been obtained with fairly high target doses of ACE inhibitors, ie, 100 to 150 mg captopril. On the assumption that low doses might be as effective as high doses while reducing side effects, low doses are commonly used in clinical practice, although the optimal dose regimen for improvement of survival remains to be defined and may differ from doses that alleviate symptoms.

In the present study, we used the rat model of chronic MI to compare two dosages of lisinopril that resulted in serum drug concentrations comparable to the drug levels seen in heart failure patients receiving a low dose of 2.5 to 5 mg/d or a high dose of 20 mg/d. The latter dose is equivalent to the target dose of enalapril used in large clinical trials. Treatment with the high dose resulted in a substantial reduction of the 1-year mortality rate, whereas the low dose did not. Importantly, the 1-year mortality rate of rats receiving high-dose lisinopril was significantly lower than the mortality rate of rats on low-dose treatment. In an additional analysis, the mortality data were stratified according to infarct size and analyzed with a Cox proportional-hazards model. In relation to the 1-year mortality of vehicle-treated infarcted rats, low-dose treatment caused a nonsignificant 19.9% risk reduction, whereas high-dose treatment resulted in a highly significant risk reduction of 71.1%. To analyze whether the effects of low-dose or high-dose lisinopril depend on infarct size, rats were classified as having small, moderate, and large infarcts. Low-dose treatment did not reduce long-term mortality in any of the three groups, whereas the high dose prolonged survival in rats with small and moderate infarcts. These findings are consistent with earlier results of Pfeffer et al, who demonstrated that a hypotensive dose of captopril reduced the long-term mortality of rats with moderate but not with large infarcts.

Plasma and Tissue ACE Activities During Chronic Lisinopril Treatment

What are the mechanisms that underlie the therapeutic efficacy of the “high” dose, and why did the “low”
dose fail to reduce long-term mortality? The rat model of chronic MI represents an animal model of chronic compensated congestive heart failure. Similar to the hormonal profile of patients with asymptomatic LV dysfunction after MI, the circulating levels of renin, ACE, and Ang II are not elevated in the chronic compensated stage of this model. In agreement with these earlier results, plasma ACE activities and plasma renin activities were similar in untreated infarcted and sham-operated rats in the present study. The tissue RAS, however, is activated in the heart of rats with chronic MI. In the heart, right ventricular and interventricular septal ACE activities, right ventricular ACE mRNA levels, and LV angiotensinogen mRNA levels are elevated in relation to infarct size. Consistent with these data, we observed a selective increase in LV ACE activity and ACE mRNA in infarcted rats. Conceivably, an upregulation of the ACE in the heart may lead to an increased intracardiac Ang I to Ang II conversion rate. Ang II generated locally may cause impairment of diastolic relaxation and might be a mediator of ventricular hypertrophy via its growth-promoting effects. In the kidneys, the expression of angiotensinogen mRNA is upregulated in relation to infarct size. The resulting increase in renal Ang II levels may contribute to sodium and fluid retention in heart failure. It has been speculated that interference with the activated tissue RAS in the heart and kidney might, in part, mediate the beneficial effects of ACE inhibitors.

Both dosages of lisinopril resulted in a profound inhibition of plasma and pulmonary ACE activities. Moreover, both dose regimens exerted a significant rightward shift of the Ang I pressure-response curve, indicating a sustained inhibition of the plasma RAS during the 1-year treatment. Our observation that low-dose lisinopril did not affect mortality after MI indicates that inhibition of the plasma and pulmonary ACE alone does not necessarily improve survival after MI in the rat. Importantly, renal ACE activity was inhibited only by high-dose lisinopril, possibly representing an important mechanism involved in the beneficial effect on survival. In the rat infarct model, inhibition of renal ACE by enalapril has been shown to be associated with a normalization of renal angiotensinogen mRNA levels and a higher daily sodium and fluid excretion. Moreover, blood pressure reduction during chronic enalapril treatment correlates with the degree of renal ACE inhibition. Consistent with these recent findings, high-dose lisinopril caused a stronger reduction in arterial blood pressure compared with low-dose treatment.

Cardiac ACE activity was not affected by long-term high-dose lisinopril therapy but was profoundly depressed after acute administration. Consistent with the present study, both captopril and enalapril failed to suppress interventricular septal ACE activity during chronic administration in the rat infarct model, although a significant inhibition after acute gavage was achieved by both agents. It should be noted that the measurement of tissue ACE activities ex vivo underestimates the degree of functional ACE inhibition in vivo. On the basis of these recent observations, it is quite likely that a partial inhibition of cardiac ACE by lisinopril (or captopril) was achieved in vivo. This assumption is consistent with recent observations that long-term ACE inhibition in the rat infarct model is associated with reduced cardiac Ang II content, whereas plasma angiotensin levels remained unchanged. However, the lack of inhibition of cardiac ACE activity ex vivo after long-term therapy in the face of profoundly depressed cardiac ACE activity ex vivo after acute administration indicates that other factors are involved in the blunted inhibition of cardiac ACE activity during long-term lisinopril therapy. Hirsch et al speculated that the cardiac ACE might be induced during chronic treatment. In fact, recent data suggest that ACE inhibitor treatment stimulates ACE gene expression in the lungs by reducing the synthesis of Ang II, thereby removing a negative feedback mechanism.

By using a competitive RT-PCR, we demonstrate that LV ACE mRNA levels are upregulated by ~80% in infarcted rats. Importantly, ACE mRNA expression correlated with ACE activity, suggesting that increased mRNA levels are translated into increased LV ACE activity in these rats. However, the expression of ACE mRNA was similar in infarcted rats treated with lisinopril and vehicle over 6 weeks. Although the present study does not show induction of ACE mRNA in the LV with long-term ACE inhibitor treatment, our findings do not exclude the possibility that a transient induction of the enzyme occurs after initiation of ACE inhibitor therapy. Studies are currently under way in our laboratory to address this issue. Conceivably, increased LV expression of ACE mRNA early after administration of ACE inhibitors may be offset during long-term therapy by sustained unloading of the LV due to systemic effects of ACE inhibitors. Indeed, the present study shows that the expression of LV ANF mRNA, a marker gene whose expression is upregulated in cardiac hypertrophy and failure, was reduced during long-term high-dose lisinopril.

**Influence of Low-Dose and High-Dose Lisinopril on Ventricular Weights and LV ANF mRNA Expression**

A reduction of LV weights was achieved by high-dose but not by low-dose lisinopril. This effect was present in analysis of the data of the 1-year treatment protocol as well as in rats treated for 6 weeks (ie, before the onset of excess mortality in the infarcted animals). This suggests that the high-dose regimen prevented the development of reactive hypertrophy of the LV. Conversely, the LV weights of placebo- and low-dose-treated infarcted rats were similar compared with sham animals, indicating the presence of reactive cardiac hypertrophy compensating for the loss of myocardium after infarction, which is replaced by scar tissue of low mass density. Thus, improved survival after MI achieved by high-dose treatment was associated with prevention of LV hypertrophy, and the failure of low-dose lisinopril to improve survival was associated with development of LV hypertrophy. Although the association does not prove a causal relation, it is tempting to speculate that reduction in LV hypertrophy during ACE inhibitor therapy is a marker for a beneficial effect on survival. Our results on the ventricular expression of ANF, an established molecular marker of LV hypertrophy and increased wall stress, would support our notion. The ANF gene expression is very low in the normal...
adult ventricle but high in the ventricles during embryonic development and in ventricular hypertrophy. An upregulation of ventricular ANF expression has been demonstrated in the rat model of chronic MI as well as in other animal models of ventricular hypertrophy. In agreement with an earlier study from our group, plasma ANF levels and LV ANF mRNA expression were elevated in infarcted rats. Plasma levels of ANF tended to decrease with lisinopril treatment. Only high-dose lisinopril treatment significantly reduced the expression of ANF mRNA in the LV, consistent with a previous study using a hypotensive dose of an ACE inhibitor. An increase in ventricular wall stress is considered to represent an important trigger mechanism for the upregulation of LV ANF mRNA expression. Since high-dose but not low-dose lisinopril caused a sustained reduction in arterial blood pressure over a period of 1 year, it is quite likely that the reduction in ANF mRNA expression was primarily attributed to LV unloading. Notably, low- and high-dose lisinopril differed in their ability to reduce arterial blood pressure, LV ANF expression, and LV hypertrophy. Therefore, the results from the present study as well as the data reported by Pfeffer et al suggest that hypotensive dosages are required so as to reduce the long-term mortality in rats with chronic MI. It should be noted that this may not necessarily be true for other animal models of heart failure or pressure overload. In rats made hypertensive by aortic banding, a reduction of LV hypertrophy and interstitial myocardial fibrosis was achieved by a very low dose of ramipril that did not affect arterial blood pressure. Thus, the relative contributions of afterload reduction and direct cardiac action for the beneficial effects of ACE inhibitors may vary depending on the animal model and/or the end point studied.

In summary, high-dose ACE inhibition, characterized by a sustained reduction of arterial blood pressure, inhibition of renin ACE activity, and reduction of LV hypertrophy, reduced long-term mortality after MI in the rat. Low doses, although exerting significant inhibition of circulating and pulmonary ACE, do not affect LV hypertrophy and long-term mortality after MI. Persistent inhibition of renal ACE may play an important role in this respect by limiting sodium retention. The present observations may have important clinical implications by providing experimental evidence for the requirement of high-dose ACE inhibition to improve survival after MI. The results of a small clinical pilot study are consistent with this notion, suggesting that high doses of captopril are more effective compared with doses that are commonly used in clinical practice with regard to improvement of functional status and survival in patients with heart failure.

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K C Wollert, R Studer, B von Bülow and H Drexler

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