Effects of Quinapril, a New Angiotensin Converting Enzyme Inhibitor, on Left Ventricular Failure and Survival in the Cardiomyopathic Hamster

Hemodynamic, Morphological, and Biochemical Correlates

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The effect of chronic therapy with quinapril on the temporal progression of left ventricular failure and survival was assessed in the CHF 146 cardiomyopathic (CM) hamster, which is an idiopathic model of congestive heart failure. Age-matched Golden Syrian (GS) hamsters served as normal controls. Quinapril was administered in the drinking water at average daily doses of 10.2, 112.4, and 222.4 mg/kg/day. In untreated CM hamsters, in vitro left ventricular performance progressively deteriorated with increasing age beginning at roughly 180 days. This decline in left ventricular performance was accompanied by a decrease in coronary flow and an increase in left ventricular volume. Administration of quinapril from 180 to 300 days of age prevented the decline of in vitro left ventricular contractile performance and coronary flow and also reduced the age-dependent increases in left ventricular volume. The cardioprotective effects of quinapril were observed at doses of 112.4 and 222.4 mg/kg/day but not at 10.2 mg/kg/day. Lung angiotensin converting enzyme activity was significantly inhibited by quinapril in GS and CM hamsters at 240 and 300 days of age at all dose levels. In contrast, significant inhibition of ventricular angiotensin converting enzyme activity was observed consistently at doses of 112.4 and 222.4 mg/kg/day quinapril but not at 10.2 mg/kg/day. In the survival protocol, CM and GS hamsters were treated with vehicle or quinapril (100 mg/kg/day) from 180 to 522 days of age. During the initial 210 days of treatment (from 180 to 390 days of age) 78.3% of the vehicle-treated CM hamsters died compared with 27.7% of quinapril-treated CM hamsters. Quinapril increased the median survival time of CM hamsters by 32.9% (112 days). It is concluded that chronic quinapril therapy exerts a significant cardioprotective effect and also increases survival. (Circulation Research 1991;68:1302–1312)

Therapy for patients with congestive heart failure is changing, and in many cases the use of vasodilators has begun to replace positive inotropic agents. Angiotensin converting enzyme (ACE) inhibitors have been shown to improve the hemodynamic status and exercise tolerance of patients with congestive heart failure. More importantly, their capacity to prolong life in patients with congestive heart failure was also recently demonstrated. Preclinically, hemodynamic improvement and prolonged survival have been demonstrated with ACE inhibitors in experimental models of heart failure involving myocardial infarction. However, the etiology of congestive heart failure is diverse, including coronary artery disease with and without myocardial infarction, hypertrophic cardiomyopathy, and idiopathic cardiomyopathy as major classifications. To date, the potential beneficial effects of ACE inhibition have not been demonstrated in an animal model of heart failure involving cardiomyopathy.

The cardiomyopathic (CM) hamster has been characterized as a hypertrophic model of cardiomyopathy, which progresses to dilated congestive heart failure in the later stages of the disease. The pathogenesis of the disease state is not fully understood, but as early as 30 days of age, focal necrosis is apparent and continues up to 4 months, resulting in extensive replacement of viable myocardium with
fibrotic connective tissue.\textsuperscript{11} Although this animal model has been used to determine the effect of various pharmacological agents, including digitalis,\textsuperscript{12} calcium channel blockers,\textsuperscript{12,13} and \(-\alpha_{1}\)-adrenergic antagonists,\textsuperscript{14} on the progression of left ventricular failure, the effects of long-term therapy with ACE inhibitors have not been studied.

Quinapril, a new ACE inhibitor, is currently undergoing clinical development for the treatment of congestive heart failure.\textsuperscript{15,16} To evaluate the potential beneficial effects of quinapril therapy in a cardiomyopathic model of congestive heart failure, the CM hamster was chronically treated with quinapril. Hemodynamic, morphological, and biochemical measurements were made during the period in which in vitro performance would normally deteriorate to assess the cardioprotective effects of such therapy. In addition, the effects of chronic quinapril therapy on survival time were assessed in a subsequent 11-month mortality study. The results of the present study provide the first report that an ACE inhibitor exerts significant cardioprotection and prolonged survival in an idiopathic model of congestive heart failure.

Materials and Methods

Animals and Experimental Design

CHF 146 CM hamsters and normal Golden Syrian (GS) hamsters (Canadian Hybrid Farms, Nova Scotia, Canada) were obtained 2–3 weeks before the start of the study to equilibrate to their diet and their surroundings. They were housed in individual cages with free access to laboratory chow (Purina, Richmond, Ind.) and were maintained on a 12-hour light/dark cycle. GS and CM hamsters were age-matched and randomly assigned to experimental groups. The study included three experimental protocols, which were carried out in succession. The purpose of the first protocol was to identify the time period at which in vitro left ventricular hemodynamic performance deteriorates in the CM hamster. The goal of the second study was to ascertain the ability of the ACE inhibitor quinapril to prevent the age-dependent changes in left ventricular performance. In the final protocol, the effects of quinapril on survival time were assessed.

For the quinapril cardioprotection protocol, we wanted to intervene with therapy in the CM hamster after the necrotic stage (with measurable impairment of left ventricular performance) but before clinical signs of congestive heart failure were present. Based on previous reports\textsuperscript{11,13} and the results of the initial protocol, an evaluation period beginning at 180 and ending at 300 days of age was chosen as the appropriate test interval. For this protocol, GS and CM hamsters were divided equally into two groups: in one group, the isolated heart preparation was used for the assessment of in vitro hemodynamic performance; in the other group, various biochemical markers were measured. The hamsters were studied at 180 days (before treatment began) and at 240 and 300 days of age (after 60 and 120 days of treatment, respectively). CM and GS hamsters were treated with one of three dose levels of quinapril or vehicle (10% honey water to offset the bitter taste of quinapril) administered in their drinking water. The doses of quinapril chosen were 0.1, 1.0, and 2.0 mg/ml. These doses were based on previous studies with captopril\textsuperscript{7,9} and a preliminary study (authors’ unpublished observations) indicating that these doses produce circulating levels of quinaprilat (the active form of quinapril) that span the range of therapeutic levels seen in patients. Vehicle and quinapril solutions were prepared daily and provided to the hamsters in 10-ml aliquots at 8:00 AM (beginning of the light cycle). The 10-ml aliquot was chosen based on a previous study in CM hamsters in which each animal drank 5–7 ml water/day.\textsuperscript{13} Consumption was monitored to calculate average daily doses. Administration of 0.1, 1.0, and 2.0 mg quinapril/ml resulted in average daily doses of 10.2, 112.4, and 222.4 mg/kg/day, respectively.

For the mortality protocol, 180-day-old CM and GS hamsters were randomly assigned to vehicle (10% honey water) or quinapril treatment groups. Based on the results from the cardioprotection protocol, 100 mg quinapril/kg/day was chosen as the appropriate dose. On a weekly basis, the hamsters were weighed, and the volume of treatment was adjusted to the average weight of the treatment group. At the time of weighing, each hamster was inspected for signs of “wet tail,” a fatal diarrhea in rodents that occurs frequently in hamsters.\textsuperscript{17} Hamsters dying with wet tail and concomitant weight loss were excluded from the study. Based on this criteria, 11 of 71 GS hamsters and 12 of 93 CM hamsters were excluded during the course of the study. In addition, three hamsters that died accidentally were excluded. The final number of hamsters in each treatment group was as follows: 43 in the CM-vehicle group, 37 in the CM-quinapril group, 31 in the GS-vehicle group, and 28 in the GS-quinapril group. The end point of the study was the death of >75% of the hamsters in the CM-quinapril treatment group.

Isolated Perfused Heart Studies

Hamsters were weighed, heparinized (1,000 units i.p.), and anesthetized with pentobarbital (50 mg/kg i.p.). Their hearts were excised and perfused by the Langendorff method at a pressure of 60 mm Hg. Aortic perfusion pressure was measured via a fluid-filled catheter (PE-100) connected to a pressure transducer (Statham P23Db, Spectramed, Oxnard, Calif.) positioned at the level of the isolated hearts. Perfusion pressure was regulated with a computer-controlled servomechanism previously described.\textsuperscript{18} Hearts were perfused with a modified Krebs-Henseleit solution containing the following (mM): NaCl 117, KCl 4.3, CaCl\(_{2}\) 3.5, K\(_2\)HPO\(_4\) 0.1, MgCl\(_{2}\)6H\(_2\)O 1.2, NaHCO\(_3\) 25, sodium EDTA 0.6, and dextrose 15. Instrumentation of the isolated hearts is illustrated in Figure 1. The hearts were paced (stimulator, model S88, Grass Instrument Co., Quincy, Mass.) at 240 beats/min via
platinum electrodes spaced 3 mm apart and placed in contact with the base of the right ventricle. The left ventricle was catheterized with a 4F catheter tip pressure transducer (Millar, Houston) via the mitral valve for the measurement of left ventricular pressure. A ligature was secured around the left atrium and pulmonary veins encompassing the pressure catheter to prevent retrograde ventricular leakage across the mitral valve. The ligature also prevented movement of the catheter during assessment of left ventricular performance. Coronary flow was measured with an ultrasonic flow sensor positioned proximal to the aortic valves (Transonic Systems, Inc., Ithaca, N.Y.). Analog signals (aortic perfusion pressure, left ventricular pressure, and coronary flow) were simultaneously recorded on a strip chart recorder and digitized with a data analyzer (Buxco Electronics, Sharon, Conn.), which yielded the following parameters: left ventricular end-diastolic pressure (LVEDP), the maximum positive first derivative of left ventricular pressure (LV +dP/dt max), and mean coronary flow. Coronary flow values were normalized to dry ventricular weight (milliliters per minute per gram dry ventricular weight). Hearts were permitted a 30-minute stabilization period before assessing left ventricular performance. Left ventricular performance was assessed by determining the ability of the isolated hearts to respond to increases in workload. Workload was increased by increasing aortic perfusion pressure from 60 to 120 mm Hg in 20–mm Hg steps (Figure 1).

Left ventricular dilation was estimated by the following procedure. The left ventricular catheter tip pressure transducer was replaced with a catheter (PE 100) filled with physiological salt solution. Coronary perfusion was stopped, and once the heart arrested, left intraventricular pressure was equilibrated to 20 mm Hg via the ventricular cannula. A ligature was secured around the base of the heart at the atrioventricular groove to prevent changes in left intraventricular volume. The atria and great vessels were trimmed, and the right ventricle was drained by an incision near the base of the right ventricle. Left ventricular wall and chamber volume was then determined by volume displacement in a calibrated chamber. Left ventricular dilation was estimated by normalizing ventricular wall and chamber volume relative to dry ventricular weight (microliters per millgram dry ventricular weight). Ventricles were dried at 100°C for 24 hours in a vacuum oven.

**Diagram showing the instrumentation used to measure in vitro hemodynamic responses of the isolated Langendorff hamster heart preparation to stepwise increases in perfusion pressure. LVP, left ventricular pressure.**

**Biochemical Measurements**

Immunoreactive plasma atrial natriuretic peptide (ANP) concentration was measured using a radioimmunoassay procedure, as previously described by Brands and Freeman. Briefly, after administration of anesthesia (50 mg/kg i.p. pentobarbital) and removal of the heart, blood was collected from the chest cavity in tubes containing EDTA (1 mg/ml blood) and centrifuged (3,000 rpm for 15 minutes). The plasma obtained was then frozen in liquid nitrogen and stored at −70°C. Immunoreactive ANP was extracted by applying 0.75–1.0 ml plasma to disposable C18 reversed-phase Bond Elut columns (3 ml/200 mg, Analyticchem International, Inc., Harbor City, Calif.) previously equilibrated with 0.1 M acetic acid. After washing with 0.1 M acetic acid, ANP was eluted with 1 ml of 50% (vol/vol) acetonitrile in 50 mM NaH2PO4, pH 7.4. The eluate containing ANP was evaporated to dryness and resuspended in 0.1 ml assay buffer immediately before assay. The extracted ANP was quantitated using a sequential-saturation, double-antibody radioimmunoassay (Peninsula Laboratories, Belmont, Calif.).

Tissue ACE activity was measured in solubilized tissue extracts from lung and ventricular muscle, using standard procedures. After administration of anesthesia (50 mg/kg i.p. pentobarbital), 0.5-g samples of the lung and the left ventricle were removed, rinsed in saline, and quick-frozen in liquid nitrogen. The tissue samples were stored at −70°C until assayed for ACE activity (within 2 months). After thawing, the tissues were blotted dry, weighed, and added to 50-ml centrifuge tubes containing 2.0 ml (heart) or 5.0 ml (lung) of “homogenization buffer,” which consisted of 20 mM Tris base, 5 mM Mg (CH3COO)2, 30 mM KCl, 250 mM sucrose, and 0.5% Triton X-100, pH 8.3. The samples were then homogenized using a polytron (Brinkmann Instruments, Inc., Westbury, N.Y.) (three 5-second passes at a setting of 8). After homogenization, the polytron probe was rinsed with an additional 2.0 or 5.0 ml of homogenization buffer. All homogenization procedures were performed at 4°C. The homogenate was stored overnight at 4°C and then centrifuged at 20,000g for 20 minutes in an RCSC centrifuge (Sorval Instruments, Wilmington, Del.). The supernatant thus obtained was used as a source of tissue ACE. ACE activity in the supernatant was determined by
measuring the conversion of [3H]hippuryl-glycine-glycine to [3H]hippuric acid using 40 μl “assay buffer” containing (mM) HEPES 50, NaCl 100, and Na₂SO₄ 600, pH 8.0. The assay medium was incubated at 37°C for 60 minutes, and the reaction was terminated by the addition of 1.0 ml of 0.1 M HCl. The [3H]hippuric acid generated was extracted with 1.0 ml ethyl acetate. Reaction conditions were adjusted to ensure that no more than 15% of the available substrate was converted to product.

Materials

All reagents used for these experiments were of the highest obtainable commercial purity. Quinapril was prepared by Warner-Lambert/Parke-Davis, Ann Arbor, Mich.²³

Statistical Analysis

Statistical differences (p<0.05) between mean values within a single hamster strain at different ages or at the same age between treatment groups were determined using analysis of variance followed by Fisher’s least significant difference test.²⁴ Significant differences at one age between strains were determined using paired, two-tailed t tests (p<0.05).²⁴ The probability of survival between vehicle- and quinapril-treated hamsters was determined using the Kaplan-Meier approach to survival distributions, the log-rank test (p<0.05).²⁵

Results

Progression of Left Ventricular Failure in CM Hamsters

Figure 2 summarizes the changes in left ventricular hemodynamic performance in response to increases
in aortic perfusion pressure for GS (panels A–C) and CM (panels D–F) hearts. GS hearts from 180-, 240-, and 300-day-old hamsters responded to increases in aortic perfusion pressure with linear increases in LV +dP/dt\textsubscript{max} (Figure 2A). Intragroup comparison of GS hearts from the three age groups indicated comparable increases in LV +dP/dt\textsubscript{max}. Hearts from 180-day-old CM hamsters also responded to increases in aortic perfusion pressure with linear increases in LV +dP/dt\textsubscript{max} (Figure 2D). Although LV +dP/dt\textsubscript{max} increased in a linear fashion, an intergroup comparison between hearts from 180-day-old GS and CM hamsters indicated a significantly lower value of LV +dP/dt\textsubscript{max} (approximately 300 mm Hg/sec) at each level of perfusion pressure for the CM hamster. Unlike GS hearts, age-dependent reductions in LV +dP/dt\textsubscript{max} were observed in the CM hamster hearts. Not only were the values of LV +dP/dt\textsubscript{max} significantly less for hearts from the 240- and 300-day-old CM hamsters, but the slope of the LV +dP/dt\textsubscript{max} response curve was depressed.

For all age groups of GS hearts, LVEDP was near 0 mm Hg over the range of aortic perfusion pressures of 60–120 mm Hg (Figure 2B). In contrast, LVEDP increased significantly for CM hearts as aortic perfusion pressure was increased (Figure 2E). Furthermore, the increases in LVEDP in response to increases in aortic perfusion pressure became progressively greater with increased age. At 300 days of age, LVEDP rose from...
FIGURE 5. Graphs showing dose-dependent effects of chronic quinapril administration on coronary flow in response to increasing aortic perfusion pressure in hearts from 240-day-old (panel A) and 300-day-old (panel B) CHF 146 cardiomyopathic hamsters. ○, Hearts from vehicle-treated hamsters; ●, hearts from hamsters administered 10.2 mg quinapril/kg/day; ▲, hearts from hamsters administered 112.4 mg quinapril/kg/day; ■, hearts from hamsters administered 222.4 mg quinapril/kg/day; d, dry ventricular weight. Symbols represent mean ± SEM of four to six separate experiments. †Significant difference between vehicle-treated hamster hearts and quinapril-treated hamster hearts at the indicated aortic perfusion pressure levels, p<0.05.

3.2 mm Hg at an aortic perfusion pressure of 60 mm Hg to 49.9 mm Hg at an aortic pressure of 120 mm Hg. Coronary flow increased in response to increases in aortic perfusion pressure for both GS and CM hearts (Figures 2C and 2F, respectively). As with LV +dP/dt\text{max} and LVEDP, coronary flow values were comparable for all age groups of GS hearts. Coronary flow values for 180-day-old CM hearts were not significantly different from values for 180-day-old GS hearts. However, coronary flow values for hearts from 240- and 300-day-old hamsters were significantly reduced compared with hearts from 180-day-old CM hamsters (Figure 2F) or with hearts from age-matched GS hamsters.

Effects of Quinapril on Left Ventricular Performance in CM and GS Hamsters

Figures 3–5 show the dose-dependent effects of quinapril treatment on in vitro left ventricular performance and coronary flow in hearts from 240- and 300-day-old CM hamsters. Quinapril treatment at doses of 112.4 and 222.4 mg/kg/day resulted in significantly greater levels of LV +dP/dt\text{max} over the range of aortic perfusion pressures (60–120 mm Hg) for both 240- and 300-day-old CM hamster hearts (Figures 3A and 3B, respectively) compared with age-matched, vehicle-treated CM hearts. At 240 days of age, the differences in LV +dP/dt\text{max} values between vehicle-treated and quinapril-treated (112.4 and 222.4 mg/kg/day) CM hearts ranged between 250 mm Hg/sec at a perfusion pressure of 60 mm Hg and 600 mm Hg/sec at 120 mm Hg perfusion pressure. At 300 days of age, the difference in LV +dP/dt\text{max} between vehicle-treated and quinapril-treated CM hamsters widened to 800–1,200 mm Hg/sec at perfusion pressures of 60–120 mm Hg. Quinapril treatment at the dose level of 10.2 mg/kg/min did not significantly improve LV +dP/dt\text{max} levels of CM hearts compared with the age-matched, vehicle-treated CM hearts.

FIGURE 6. Graphs showing dose-dependent effects of chronic quinapril administration on ventricular wall and chamber volume of Golden Syrian (solid bars) and CHF 146 cardiomyopathic (slashed bars) hamsters at 240 (panel A) and 300 days of age (panel B). Bars represent mean ± SEM of three to seven ventricles. *Significant difference from age-matched Golden Syrian controls, p<0.05. †Significant difference from age-matched Golden Syrian controls, p<0.07.
Figure 4 shows the effects of quinapril treatment on the response of LVEDP to increases in aortic perfusion pressure. Quinapril treatment at 112.4 and 222.4 mg/kg/day resulted in significantly lower levels of LVEDP for 240- and 300-day-old CM hamster hearts compared with vehicle-treated CM hamster hearts. At these doses of quinapril, the average level of LVEDP at a perfusion pressure of 120 mm Hg was between 8 and 10 mm Hg for 240-day-old CM hearts (Figure 4A) and between 13 and 15 mm Hg for 300-day-old CM hearts (Figure 4B). In comparison, LVEDP for vehicle-treated CM hearts at 120 mm Hg perfusion pressure was 40 and 60 mm Hg for 240- and 300-day-old CM hearts, respectively. There were no significant differences in LVEDP levels between hearts from CM hamsters treated with 10.2 mg/kg/day quinapril and vehicle.

Coronary flow levels were significantly greater in CM hearts treated with quinapril at dose levels of 112.4 and 222.4 mg/kg/day compared with age-matched, vehicle-treated CM hearts (Figure 5). The degree of improvement in coronary flow between these treatment groups varied according to age and aortic perfusion pressure (see Figures 5A and 5B for significant points). There were no significant differences in coronary flow levels between hearts from CM hamsters treated with 10.2 mg/kg/day quinapril and those treated with vehicle.

For GS hamsters, chronic quinapril treatment had no significant effect on LV + dP/dt max or LVEDP at any dose level (data not shown). However, coronary flow levels in hearts from GS hamsters were significantly increased by quinapril treatment at 112.4 and 222.4 mg/kg/day. This increase was observed at some but not all aortic perfusion pressure levels (data not shown).

### Effects of Quinapril on Ventricular Morphology

In general, ventricular mass (normalized to dry ventricular weight/body weight) was not significantly different between GS and CM hamsters in either the vehicle or the quinapril-treated groups (Table 1). Although little difference in ventricular mass was noted, significant differences were observed in ventricular volume (microliters of water displaced per milligram dry ventricular weight) between vehicle-treated GS and CM hamsters (Figures 6A and 6B). In con-

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**Table 1. Ventricular Mass in Control Golden Syrian and CHF 146 Cardiomyopathic Hamsters**

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>240 days of age</th>
<th>300 days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Golden Syrian</td>
<td>Cardiomyopathic</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.98±0.03 (5)</td>
<td>0.90±0.04 (7)</td>
</tr>
<tr>
<td>Quinapril</td>
<td></td>
<td></td>
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<tr>
<td>10.2 mg/kg/day</td>
<td>0.93±0.03 (6)</td>
<td>0.87±0.02 (6)</td>
</tr>
<tr>
<td>112.4 mg/kg/day</td>
<td>0.88±0.04 (5)</td>
<td>0.87±0.04 (5)</td>
</tr>
<tr>
<td>222.4 mg/kg/day</td>
<td>0.83±0.04† (6)</td>
<td>0.84±0.02 (6)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Values in parentheses refer to the number of hamsters studied in each group.

*Significant difference from age-matched Golden Syrian control hamsters, p<0.05.
†Significant difference from vehicle-treated control hamsters within the same strain, p<0.05.

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**Table 2. Tissue Angiotensin Converting Enzyme Activity in Control Golden Syrian and CHF 146 Cardiomyopathic Hamsters**

<table>
<thead>
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<th>Treatment group</th>
<th>180 days of age</th>
<th>240 days of age</th>
<th>300 days of age</th>
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<tr>
<td></td>
<td>Golden Syrian</td>
<td>Cardiomyopathic</td>
<td>Golden Syrian</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>18,884±1,810 (8)</td>
<td>21,929±1,103 (8)</td>
<td>13,685±1,313 (6)</td>
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<tr>
<td>Quinapril</td>
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<td></td>
</tr>
<tr>
<td>10.2 mg/kg/day</td>
<td>. . .</td>
<td>1,564±214* (5)</td>
<td>1,343±226* (5)</td>
</tr>
<tr>
<td>112.4 mg/kg/day</td>
<td>. . .</td>
<td>760±218* (4)</td>
<td>908±241* (6)</td>
</tr>
<tr>
<td>222.4 mg/kg/day</td>
<td>. . .</td>
<td>349±78* (7)</td>
<td>451±108* (6)</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vehicle</td>
<td>53.8±11.8 (6)</td>
<td>128.4±24.4† (8)</td>
<td>87.0±3.7 (4)</td>
</tr>
<tr>
<td>Quinapril</td>
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<td></td>
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<tr>
<td>10.2 mg/kg/day</td>
<td>. . .</td>
<td>21.3±14.0* (5)</td>
<td>32.9±12.0* (5)</td>
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<tr>
<td>112.4 mg/kg/day</td>
<td>. . .</td>
<td>14.8±7.7* (4)</td>
<td>29.1±3.8* (6)</td>
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<tr>
<td>222.4 mg/kg/day</td>
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<td>12.2±6.6* (6)</td>
<td>14.8±6.6* (6)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Values in parentheses refer to the number of hamsters studied in each group. ACE, angiotensin converting enzyme.

*Significant difference from vehicle-treated control hamsters within the same strain, p<0.05.
†Significant difference from age-matched, Golden Syrian control hamsters, p<0.05.
TABLE 3. Plasma Atrial Natriuretic Peptide Concentration in Control Golden Syrian and CHF 146 Cardiomyopathic Hamsters

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Golden Syrian (pg/ml)</th>
<th>Cardiomyopathic (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180 days of age</td>
<td>240 days of age</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>10.2±2.4 (8)</td>
<td>9.9±1.6 (3)</td>
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<tr>
<td>Quinapril</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.2 mg/kg/day</td>
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</tr>
<tr>
<td>112.4 mg/kg/day</td>
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</tr>
<tr>
<td>222.4 mg/kg/day</td>
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</tbody>
</table>

Values are mean±SEM. Values in parentheses refer to the number of hamsters studied in each group. ANP, atrial natriuretic peptide.
*Significant difference from Golden Syrian control hamsters, p<0.05.
†Significant difference from vehicle-treated control hamsters within the same strain, p<0.05.

Effects of Quinapril on Tissue ACE Activity

ACE activity was determined for lung and ventricular tissue (Table 2), which was collected 4–6 hours after the hamsters had been provided with their respective treatment. Lung ACE activity was not significantly different between GS and CM vehicle-treated hamsters at 180, 240, or 300 days of age. Despite a lack of statistical significance, at 300 days, lung ACE activity was 38% greater in vehicle-treated CM hamsters compared with age-matched GS hamsters (Table 2). Quinapril, at all dose levels, significantly inhibited lung ACE activity in 240- and 300-day-old GS and CM hamsters. At the lower dose level of 10.2 mg/kg/day, lung ACE was inhibited by >85%, independent of hamster strain or age group. Ventricular ACE activity was generally greater (34.6–114.6%) in vehicle-treated CM hamsters compared with vehicle-treated GS hamsters for all age groups. However, statistical significance was not achieved in all age groups. In 240-day-old hamsters, quinapril treatment significantly inhibited ventricular ACE activity at all dose levels. In 300-day-old hamsters, the ventricular ACE activity of CM hamsters was significantly inhibited at quinapril doses of 112.4 and 222.4 mg/kg/day, but not at 10.2 mg/kg/day.

Effects of Quinapril on Plasma ANP Levels

Plasma ANP concentrations were significantly greater in CM hamsters compared with GS hamsters for 180-, 240-, and 300-day-old hamsters (Table 3). The elevated levels of plasma ANP for CM hamsters did not change significantly over the 120-day test period. Quinapril treatment had variable effects on ANP concentrations in CM hamsters. At 240 days of age, quinapril did not significantly affect plasma ANP concentrations in CM hamsters compared with vehicle-treated CM hamsters. At 300 days of age, plasma ANP concentrations of CM hamsters treated with 112.4 mg/kg/day quinapril were significantly less than those of vehicle-treated CM hamsters. In contrast, ANP levels in CM hamsters treated with 222.4 mg/kg/day quinapril were not significantly different from levels in the vehicle-treated CM hamsters.

Effects of Quinapril on Survival

The survival study ran for 342 days, beginning at approximately 180 days of age and ending at approximately 522 days of age. Figure 7 shows the effects of quinapril treatment on survival in CM hamsters. From 212 to 391 days of age, the mortality rate for quinapril-treated CM hamsters was significantly less (p<0.0001) than that for vehicle-treated CM hamsters. The probability of survival at 391 days of age was 27.7% for vehicle-treated CM hamsters compared with 78.3% for quinapril-treated CM hamsters. After 391 days of age, the mortality rate for quinapril-treated CM hamsters paralleled that of vehicle-treated CM hamsters. The median probability of survival was 340 days of age for vehicle-treated CM hamsters compared with 452 days of age for quinapril-treated CM hamsters. This represents an average increase in survival time of 112 days or 32.9% with quinapril treatment. At the conclusion of the study (approximately 522 days of age), eight of 37
quinapril-treated CM hamsters were alive compared with one of 43 vehicle-treated CM hamsters.

Only two vehicle-treated GS hamsters and three quinapril-treated GS hamsters died over the 342-day test period (data not shown). The probability of survival was not statistically significant between the two treatment groups.

Discussion

The disease process in the CM hamster has been separated into four temporal phases: preneecrotic, necrotic or myolytic, hypertrophic, and terminal. The necrotic stage begins at 30–35 days of age and continues for 3–4 months. This is followed by a hypertrophic and healing stage and the beginnings of ventricular dilation. In the final stages, from 240 to 360 days of age, ventricular dilation continues, and clinical signs of congestive heart failure appear, including peripheral and pulmonary edema and decreases in physical activity. Since changes in the time course of these stages has been noted after generations of inbreeding, an initial series of experiments were performed to document the temporal progression of left ventricular failure in the CM hamster. In a subsequent series of experiments, the ability of quinapril therapy to prevent these changes was evaluated.

Left ventricular performance was assessed by determining the ability of the isolated hearts to respond to increases in workload. Workload was increased by increasing aortic perfusion pressure from 60 to 120 mm Hg in 20–mm Hg steps. Previous studies in CM hamsters have shown that increasing coronary perfusion pressure increases ventricular wall thickness through an “erection” or hydraulic effect and that the increases in left ventricular performance result from mechanical stretch of the myofibrils at end diastole. In our test model, mechanical stretch due to increases in coronary perfusion pressure could result from both the erectile effect and from increases in LVEDP.

At 180 days of age, left ventricular responses to increases in aortic perfusion pressure were qualitatively similar between GS and CM hamsters, with near linear increases in $LV +dP/dt_{max}$. Although the hearts from 180-day-old CM hamsters responded in a manner similar to normal GS hearts, the absolute level of $LV +dP/dt_{max}$ obtained at each incremental increase in perfusion pressure was significantly less. This was probably due to the extensive number of necrotic lesions in CM hearts present at this age.

At 240 and 300 days of age, the left ventricular responses to increases in aortic perfusion pressure were no longer comparable between GS and CM hamsters. The responses in $LV +dP/dt_{max}$ and LVEDP were unchanged for GS hamsters between 180 and 300 days, whereas for CM hamsters, responses in $LV +dP/dt_{max}$ were significantly depressed and nearly flat, and LVEDP increased progressively throughout the entire aortic perfusion pressure range. The results of these experiments are consistent with a previous study that indicates that the left ventricle of the CM hamster heart has a diminished ability to respond to increases in aortic perfusion pressure.

Coronary flow values were not significantly different between 180-day-old GS and CM hamster hearts. However, coronary flow values were significantly less for older (240- and 300-day-old) CM hearts. This age-dependent decrease in coronary perfusion coincided with the age-dependent deterioration in left ventricular performance and is consistent with previous findings. It is not clear from these data if the deterioration in left ventricular performance and the decreases in coronary perfusion are dependent events or if they occur independently in response to the heart failure process. Perivascular fibrosis and calcified lesion, anticipated at this stage of the cardiomyopathy, would be expected to decrease coronary perfusion as well as impair ventricular stiffness and function. In addition, high levels of LVEDP may have contributed to the decrease in coronary perfusion due to compression of subendocardial capillaries.

In the second part of the investigation, the effects of quinapril on the progression of left ventricle failure were evaluated. Based on the results of the initial protocol, quinapril therapy was started at 180 days of age in three groups of CM hamsters at average daily doses of 10.2, 112.4, and 222.4 mg/kg. A fourth group of CM hamsters was treated with vehicle; these hamsters served as controls. Consistent with the initial protocol, left ventricular performance progressively deteriorated in the hearts of vehicle-treated CM hamsters between 180 and 300 days of age. Quinapril therapy at the low dose level of 10.2 mg/kg/day did not significantly affect this progression. However, at the highest dose levels of 112.4 and 222.4 mg/kg/day, quinapril significantly prevented the age-dependent decline in $LV +dP/dt_{max}$ and significantly prevented the age-dependent increase in LVEDP. These results indicate that quinapril treatment preserves the ability of the left ventricle to respond to the effect of increasing coronary perfusion pressure and suggest a preserved ability of the heart to respond to myofibril stretch.

Consistent with the initial protocol, coronary flow was significantly less in CM hearts compared with GS hearts from 240- and 300-day-old hamsters treated with vehicle. Quinapril treatment at the daily dose levels of 112.4 and 222.4 mg/kg significantly increased coronary flow in CM hearts. The dose-dependent effects of quinapril on coronary flow correlated with the beneficial effects of left ventricular performance and lower levels of LVEDP. However, quinapril also significantly increased the coronary flow of GS hearts, suggesting a direct effect on coronary flow independent of left ventricular performance. In a previous study with the ACE inhibitor captopril, both sham and heart-failure animals had increases in coronary flow. Therefore, it is possible that quinapril preserved coronary flow in CM hearts.
by preserving left ventricular performance and also by exerting a direct action on the coronary vasculature. It is tempting to speculate that quinapril may have affected the process of reparative fibrosis and thereby improved ventricular function and coronary perfusion. Although we have no direct evidence for this at this time, Michel et al.34 recently demonstrated that ACE inhibition could prevent increases in the volume density of collagen in infarcted rats.

Ventricular dilation is characteristic of the CM hamster beginning in the third stage and continuing through the fourth and terminal stage of the disease.31 In this study, ventricular dilation was evident in vehicle-treated 240- and 300-day-old CM hamster hearts from a greater ventricular wall and chamber volume, without a significant difference in ventricular mass, compared with age-matched GS hamster hearts. Another indication of the beneficial effects of chronic quinapril treatment in the CM hamster was the ability of quinapril to prevent increases in ventricular wall and chamber volume. With quinapril treatment at the daily doses of 112.4 and 222.4 mg/kg/day, CM ventricular wall and chamber volume was no longer significantly different from GS ventricular wall and chamber volume. As with left ventricular performance, quinapril's effects on ventricular wall and chamber volume were dose dependent, with significant protection occurring at 112.4 and 222.4 mg/kg/day but not at 10.2 mg/kg/day.

The renin-angiotensin system is activated above normal levels in patients with congestive heart failure.35 In the present study, tissue ACE activity levels were greater in CM versus GS hamsters, suggesting that in this animal model of idiopathic congestive heart failure the renin-angiotensin system is also activated above normal levels. In general, quinapril inhibited lung and ventricular ACE in a dose-dependent manner. For lung tissue, 85–90% of the ACE activity was inhibited at the dose level of 10.2 mg/kg/day. Inhibition of ventricular ACE was much more variable at the low dose of quinapril (10.2 mg/kg/day), ranging from 72% in 240-day-old CM to only 16% in 300-day-old CM hamsters. In contrast, the dose of 112.4 mg/kg/day quinapril produced a consistent inhibition of ventricular ACE ranging from 75% to 84%. The dose level of 112.4 mg/kg/day was also the level at which quinapril prevented the progression of left ventricular failure and left ventricular dilation. Collectively, these results suggest a better correlation between inhibition of ventricular ACE and cardioprotection than between lung ACE and cardioprotection. However, an effect of quinapril on ACE activity in more specific tissue compartments (e.g., vascular smooth muscle, cardiac interstitial, or cardiovascular nervous tissue) may provide a better correlation between ACE inhibition and cardioprotection. Clearly, additional studies will be necessary before any conclusions can be made on the specific tissues involved in quinapril's cardioprotective action.

Consistent with previous findings,36 plasma ANP concentration was severalfold greater in CM hamsters compared with normal GS hamsters. This was true for all age groups, and in general, the ANP concentration in CM hamsters was approximately the same independent of age. These results indicate that plasma ANP concentration was stable, albeit elevated, in the CM hamster during a time when left ventricular performance was progressively deteriorating with age. This apparent dissociation between plasma ANP concentration and severity of left ventricular failure has previously been demonstrated in the CM hamster.36 It appears that plasma ANP increases in the CM hamster during the compensatory (hypertrophic) phase and remains elevated during the development of heart failure and then decreases slightly at the end stage of the disease just before death.37 Quinapril therapy did not reverse the preexisting (180 days of age) high levels of plasma ANP in the CM hamster. This would suggest that the amount of left ventricular impairment in 180-day-old CM hamsters was sufficient to result in elevated ANP concentrations in the plasma. Although quinapril prevented any further decline in ventricular performance beyond 180 days of age, it did not reverse the preexisting decrement in left ventricular performance, which may explain its lack of consistent effect on elevated plasma ANP concentrations in CM hamsters.

In addition to preventing the progression of left ventricular failure, quinapril increased the median probability of survival of CM hamsters by 32.9%. Quinapril's major effect on survival occurred during the first 210 days of treatment, after which the mortality rates were comparable between treatment groups. The beneficial effect on survival occurred at a time when quinapril prevented the progression of left ventricular failure. Although a cause and effect relation cannot be established from these data alone, the temporal association between improved ventricular performance and a decreased death rate suggests that quinapril prolonged survival by preventing the progression of left ventricular failure.

In summary, in vitro left ventricular performance progressively deteriorated in untreated CM hamsters with increasing age beginning at 180 days of age. This progressive decline in left ventricular performance was accompanied by decreases in coronary flow and increases in left ventricular volume. Administration of quinapril from 180 to 300 days of age prevented the decline of in vitro left ventricular contractile performance and coronary flow and also reduced the increases in left ventricular volume. In addition, chronic quinapril therapy prolonged median survival by 32.9%. It is concluded that chronic quinapril therapy produces significant cardioprotection and markedly prolongs survival in the CM hamster, an idiopathic model of congestive heart failure.

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References


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