Chronic AT<sub>1</sub> receptor blockade and angiotensin-converting enzyme (ACE) inhibition in (CHF 146) cardiomyopathic hamsters: effects on cardiac hypertrophy and survival

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Abstract

Objective: We have reported that angiotensin II AT<sub>1</sub> receptors are upregulated and that there are no AT<sub>2</sub> receptors in the ventricles of cardiomyopathic hamsters. Since the upregulation was present even when no histological lesions were detectable, these results suggested that angiotensin II plays a role in the genesis/maintenance of this pathology. A survival study was conducted to compare the effects of an angiotensin II AT<sub>1</sub> receptor antagonist, losartan (L), to those of a placebo (P). Since the angiotensin-converting enzyme (ACE) inhibitor quinapril (Q) has been shown to have beneficial effects in this animal model, a Q group was included.

Methods: Male Syrian cardiomyopathic hamsters (CHF 146, n=360) were orally administered P, low- (30 mg/kg/day) or high-dose (100 mg/kg/day) L, or Q (100 mg/kg/day), starting at day 50 of life. Inbred control hamsters (CHF 148, n=180) were treated with P or L (100 mg/kg/day) as controls. Animals were sacrificed at intervals to evaluate cardiac hypertrophy. Kaplan–Meier analysis was performed to assess differences in survival.

Results: High-dose L had no effects on the survival of control hamsters. There was an unexpected dose-dependent decrease in the survival of cardiomyopathics treated with L (low-dose, P=0.14; high-dose, P=0.0015) compared to an increase with Q (P=0.0003). Cardiac hypertrophy compared to P was increased with L but significantly decreased with Q in cardiomyopathics.

Conclusions: In this model, losartan did not improve survival compared to placebo and quinapril and, if anything, increased mortality. Our results suggest that AT<sub>1</sub> receptor antagonists and ACE inhibitors are not necessarily equivalent or interchangeable in terms of their effects on cardiac hypertrophy and survival in selected progressive heart failure models. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: ACE inhibitors; Cardiomyopathy; Hypertrophy; AT<sub>1</sub> receptors; Renin–angiotensin system

1. Introduction

Syrian golden hamsters of the cardiomyopathic strain have a non-pressure-overload cardiac disease that originates from a genetically transmitted metabolic anomaly that induces degenerative lesions in all striated muscles, with particular consistency and intensity in the heart [1]. The animals have a common defect in a gene for delta-sarcoglycan, a dystrophin-associated glycoprotein, which is functionally involved in stabilizing sarcolemma [2]. The development of the pathology is characterized by the occurrence of focal myocardial degeneration and it can be divided into four temporal phases: prenecrotic (25–30 days), necrotic (70–75 days), hypertrophic (125–150 days) with progressing dilatation (225–250 days), and severe heart failure (325–350 days) [3]. The clinical course and pathological aspects of the hamster’s chronic cardiac condition resemble hypertrophic cardiomyopathy observed in clinics. As in humans, the resulting depression of cardiac function is associated with a significant decrease in life expectancy.

Compared with normal animals, it has been shown that the cardiomyopathic hamsters display an activated renin–angiotensin system, which is characterized by higher plasma and ventricular angiotensin II concentrations, as well as higher ventricular angiotensin-converting enzyme
(ACE) activity [4–6]. Shiota et al. [7] have also reported that heart chymase is activated in the cardiomyopathic hamster during the necrotic and hypertrophic stages. It has been demonstrated that ACE inhibitors, such as quinapril, cilazapril, imidapril, indopril, enalapril and captopril, improve myocardial collagen metabolism and decrease cardiac remodeling [8–10], preserve contractile function [4,5,11], prevent the progression of left ventricular failure and/or increase the probability of survival [4,12,13] of these animals.

Many studies have demonstrated that angiotensin II, via AT$_1$ receptors, stimulates myocardial cell hypertrophy as well as growth of the cardiac interstitium [4,14]. The cardioprotective effect of chronic ACE inhibition has been observed in several hypertrophic experimental models [15–17] and in human progressive cardiac failure [18,19], has been attributed to the inhibition of cardiac ACE activity and mainly to the resulting decrease in AT$_1$ receptor activation. Studies from our laboratory have shown significant increases in ventricular angiotensin II AT$_1$ receptor expression in cardiomyopathic hamsters, whereas AT$_2$ receptors have not been detected [20]. Since the upregulation was present before the appearance of the cardiac histological lesions, this strongly suggested that the renin–angiotensin system and, more specifically, the AT$_1$ receptors, are involved in the development and/or the maintenance of the cardiomyopathy in Syrian hamsters. In this case, since it has been established that angiotensin II might be produced in the heart of hamsters via alternate pathways that are independent of the converting enzyme [7], a specific blockade of AT$_1$ receptors should offer greater protection than the inhibition of the converting enzyme.

The first aim of this study was therefore to evaluate the effects of a chronic blockade of AT$_1$ receptors on the survival of cardiomyopathic hamsters compared with those of a placebo as well as of a chronic ACE inhibition used as a positive control. The second aim was to correlate the animals’ survival with the development of the cardiac histological lesions and/or the degree of ventricular hypertrophy.

2. Methods

2.1. Materials

Losartan was supplied by Merck (Rahway, NJ, USA) and quinapril by Parke-Davis (Ann Arbor, MI, USA).

2.2. Animals and experimental groups

Male Syrian inbred control (CHF 148, $n=180$) and cardiomyopathic (CHF 146, $n=360$) hamsters, aged 40 days, were purchased from the Canadian Hybrid Farm (Nova Scotia, Canada). The animals were housed individually in a temperature-controlled room, with a 6:00 a.m. to 6:00 p.m. light–dark cycle. The animals were given a regular rat chow and had access to tap water ad libitum. At 50 days of age, the hamsters were equally divided ($n=90$) and randomly assigned to one of the experimental groups (Fig. 1).

2.3. Drug administration

The low-dose of losartan (30 mg/kg/day) was determined as a dose that significantly shifts to the right the angiotensin II pressor dose–response curves obtained in anesthetized hamsters [21]. Fig. 2 presents the results obtained in untreated animals as well as in treated hamsters 24-h following oral administration of a single dose of losartan (30 mg/kg). The dose–response curves were statistically analyzed using AllFit for Windows [22] and indicate a significant ($P<0.001$) blockade of AT$_1$ receptors. A high-dose of losartan (100 mg/kg/day) was administered to eliminate false negative results (data not
shown). To offset the bitter taste of losartan, the powder was dissolved in a flavoured commercial jelly (pH adjusted to 7.5). The placebo (jelly alone) and the drug were administered orally between 8:00 and 10:00 a.m. everyday, in 200 μl aliquots using a micropipette.

The dose of quinapril (100 mg/kg/day) was chosen on the basis of the results of Haleen et al. [4] who demonstrated that, at this dosage, quinapril significantly prevented the progression of left ventricular failure and increased the probability of survival of cardiomyopathic hamsters. The powder was dissolved and administered in drinking water that was sweetened with a sugar alternative (1%). The concentration of the solution was determined based on a previous study in which cardiomyopathic hamsters drank 5–7 ml water/day [23]. The solutions were prepared daily and given to the hamsters at 8:00 a.m.

All animals were weighed every two weeks and the concentration of drugs was adjusted to the average weight of the treatment group.

2.4. Survival

Of the 90 hamsters in each group, 12 animals were sacrificed at 175 and 325 days of age for the evaluation of cardiac histological lesions and hypertrophy. Twelve inbred control and 12 cardiomyopathic hamsters were also sacrificed at 50 days of age for heart and body weight baseline determinations.

Of the remaining animals (66/group), the hamsters that died from non-cardiac causes (wet tail, accident, etc.) were excluded from the survival study. Based on these criteria, the final number of hamsters in each treatment group was as follows: 61 in the inbred controls–placebo group; 63 in the inbred controls–losartan (100 mg/kg/day) group; 65 in the cardiomyopathics–placebo group; 65 in the cardiomyopathics–losartan (30 mg/kg/day) group; 64 in the cardiomyopathics–losartan (100 mg/kg/day) group and 64 in the cardiomyopathics–quinapril group.

Cardiac mortality was either spontaneous or provoked by the sacrifice of the animal when the following serious signs of distress were all present (peripheral edema, dyspnea, lethargy, coldness and water consumption ≤3 ml/day). The decision to sacrifice an animal when it was clear that survival was only a question of a few hours was dictated by our Deontological Committee in order to eliminate unnecessary suffering. In both cases (spontaneous or provoked), a gross necropsy was always performed to confirm the presence of the characteristic symptoms of heart failure (ascites, pleural effusion, hepatomegaly/hepatic congestion, ventricular hypertrophy and/or dilatation). The end point of the study was the death of ≥50% of the placebo-treated cardiomyopathic hamsters as well as the confirmation of a significant difference between cardiomyopathic placebo- and quinapril-treated groups. All procedures for animal experimentation conformed to the guidelines of the Canadian Council for Animal Care and were monitored by an institutional animal care committee.

The probability of survival of placebo-, losartan- (low- and high-dose) and quinapril-treated hamsters was determined using the Kaplan–Meier approach to survival distributions, the log-rank test.

2.5. Cardiac histological lesions and hypertrophy

As already mentioned, 12 hamsters per group were sacrificed at 175 days of age and 12 at 325 days of age. The animals were anesthetized with thiopental (50 mg/kg, i.p.). Their hearts were rapidly removed and allowed to beat in ice-cold PBS. After they were washed, the left and right ventricles were dissected, weighed and cut in half between the base and apex with a disposable blade. Ventrices were fixed in 10% neutral formalin for 24–48 h. Sections (7 μm) were cut and stained using the von Kossa technique for the evaluation of calcification and the Masson’s Trichrome technique for the evaluation of fibrosis.

The quantification of the lesions was done on the septum and left ventricular free walls of 325-day-old hamsters. A four-grade semi-quantitative scoring method was used to characterize the intensity and extent of tissular alterations. The scores, which were attributed according to the intensity and extent of the lesions, were expressed as very slight, slight, moderate or severe. The quantification was performed by an independent blinded pathologist (ClinTrial BioRecherches, Montréal, Canada).

Cardiac hypertrophy was evaluated in 175- and 325-day-old animals as the ventricle–body weight ratio. Data are presented as mean±SEM. Differences between groups were determined using two-way analysis of variance and Bonferroni t-tests. The critical level of significance was set at P≤0.05.

2.6. Plasma biochemical analyses

Plasma sodium, potassium, ALT (alanine transaminase) and creatinine concentrations were evaluated in 450-day-old inbred control hamsters as well as in cardiomyopathic hamsters at end-stage of failure (≥450 day-old, n=15/group). Routine laboratory tests were performed at Sainte-Justine Hospital. Plasma sodium and potassium concentrations were analyzed with selective electrodes (CX-7 Analysor, Beckman). Plasma ALT activity was measured by an enzymatic rate method using ALT reagent (Synchron CX Systems). Plasma creatinine concentrations were determined by means of the Jaffé rate method (Synchron CX Systems). Data are presented as mean±SEM. Differences between groups were determined using two-way analysis of variance and Bonferroni t-tests. The critical level of significance was set at P≤0.05.
2.7. Hemodynamic study

As hemodynamic parameters were not monitored during the survival study, the contribution of peripheral effects of the drugs on blood pressure and heart rate is unknown. Since it is well established that cardiomyopathic hamsters have low blood pressure [24,25], we believed that additional preload and afterload reductions would have provided little effect. Nevertheless, in order to rule out this possibility, an a posteriori hemodynamic study was performed. To do so, 350-day-old inbred control (n=14) and cardiomyopathic (n=21) hamsters were equally divided and randomly assigned to one of the experimental groups: inbred controls—placebo; inbred controls—losartan (100 mg/kg/day); cardiomyopathics—placebo; cardiomyopathics—losartan (100 mg/kg/day) and quinapril (100 mg/kg/day). The treatments were administered for three weeks.

On study day, the hamsters were anesthetized with a mixture of ketamine/xylazine (90 and 5 mg/kg i.m., respectively) and supplemented with the anesthetic when required. A polyethylene catheter (PE 50) filled with heparin sodium (1000 U/ml) was inserted into the right carotid artery and pushed into the aorta to continuously monitor the arterial blood pressure with a transducer connected to a blood pressure analyzer (Harvard apparatus). At the beginning of each experiment, an average equilibration period of 30 min was allowed to ensure stabilization of the preparation. Systolic and diastolic blood pressures and heart rate were then monitored. Data are presented as mean±SEM. Differences between groups were determined using two-way analysis of variance and Bonferroni t-tests. The critical level of significance was set at \( P<0.05 \).

3. Results

3.1. Effects of treatments on survival

The survival study ran for 490 days, beginning with 50-day-old animals and ending at 540 days of age. A total of 29 (six spontaneous and 23 provoked), 39 (seven spontaneous and 32 provoked), 43 (three spontaneous and 40 provoked) and ten (two spontaneous and eight provoked) cardiac deaths were noted in the cardiomyopathic groups treated with the placebo, losartan (30 mg/kg/day), losartan (100 mg/kg/day) or quinapril, respectively. Fig. 3 presents the probability of survival for the four cardiomyopathic groups only, since no cardiac deaths were observed in the inbred control groups treated with either the placebo or the high-dose of losartan.

Results for the cardiomyopathic hamsters treated with the placebo represent a typical survival curve for these animals and correspond to a median probability of survival of 489 days. As already reported by Haleen et al. [4], quinapril significantly increased the life expectancy, with a median probability of survival of more than 540 days. In fact, when the study was stopped, 77% of the hamsters included in the quinapril group were still alive. An unexpected result was that low- and high-doses of losartan decreased life expectancy when compared with the placebo \( (P=0.14 \text{ and } P=0.0015, \text{ respectively}) \) as well as with quinapril \( (P=0.0001 \text{ for both doses of losartan}) \). The resulting median probabilities of survival were of 449 days for the low-dose of losartan and of 433 days for the high-dose.

3.2. Effect of treatments on cardiac histological lesions

To explain or correlate the results obtained in the survival study, ventricular histological lesions and hypertrophy were evaluated. Table 1 shows the incidence and intensity of calcification and fibrosis in the septum and left ventricular free walls of 325-day-old inbred control and cardiomyopathic hamsters.

Myocardial calcification lesions were absent in both groups of inbred control hamsters whereas, as expected, the occurrence of these lesions was significantly increased in the cardiomyopathic animals. In all of the cardiomyopathic groups, the severity and extent of the lesions appear to be mainly of slight/moderate intensity, with an incidence independent of the treatment administered.

Myocardial fibrosis was almost absent in both groups of inbred control hamsters, with the exception of one hamster in which non-significant lesions of very slight intensity were observed. As expected, the intensity and extent of fibrotic lesions were significantly increased in the cardiomyopathic animals. In all of the cardiomyopathic groups, the severity of the lesions appears to be mainly of slight/moderate intensity, with an incidence independent of the treatment administered.
Table 1
Incidence and intensity of calcification and fibrosis in septum and left ventricular free walls of inbred control and cardiomyopathic hamsters of 325 days-of-age

<table>
<thead>
<tr>
<th></th>
<th>Inbred controls</th>
<th>Cardiomyopathics</th>
<th>Quinapril (100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Losartan (100 mg)</td>
<td>Placebo</td>
</tr>
<tr>
<td>n=</td>
<td>11</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Calcification/fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very slight</td>
<td>0/0</td>
<td>0/1</td>
<td>4/0</td>
</tr>
<tr>
<td>Slight</td>
<td>0/0</td>
<td>0/0</td>
<td>5/3</td>
</tr>
<tr>
<td>Moderate</td>
<td>0/0</td>
<td>0/0</td>
<td>1/8</td>
</tr>
<tr>
<td>Severe</td>
<td>0/0</td>
<td>0/0</td>
<td>1/0</td>
</tr>
<tr>
<td>Total with lesions</td>
<td>0/0</td>
<td>0/1</td>
<td>11/11</td>
</tr>
</tbody>
</table>

3.3. Effect of treatments on cardiac hypertrophy

Fig. 4 presents the body weights measured in 50-, 175- and 325-day-old animals. At 175 and 325 days of age, inbred controls presented significant increases compared with the cardiomyopathic groups. In these animals, the high-dose of losartan had no effect. Cardiac hypertrophy was determined as the ventricle–body weight ratio of 175- and 325-day-old hamsters. Fig. 5 presents the values obtained at 325 days of age in both groups of animals. A similar pattern was already present at 175 days of age, although significance was not reached (data not shown). The high-dose of losartan had no effect in inbred control animals. As expected, a significant increase was observed in the placebo-treated cardiomyopathic group, confirming the development of cardiac hypertrophy. This increase was not reversed and was even accentuated by both doses of losartan. In contrast, quinapril significantly decreased the ventricle–body weight ratio when compared with both losartan groups and the placebo group.

3.4. Plasma analyses

Table 2 presents the plasma sodium, potassium, ALT and creatinine concentrations evaluated in 450-day-old inbred control hamsters and cardiomyopathic hamsters at end-stage of failure (≈ 450-day-old). The high-dose of losartan had no effects on these parameters in inbred control animals. Compared with inbred control groups, significant increases in sodium, potassium and ALT concentrations were found in the cardiomyopathic group treated with the placebo, whereas no differences were noted in the creatinine concentrations. Losartan had no effects on these values, with the exception of a significant increase in creatinine concentrations related to the administration of the high dose. Quinapril significantly decreased the sodium levels and significantly increased the potassium levels in the placebo-treated cardiomyopathic group.
Pressor and chronotropic effects of losartan and quinapril in 350-day-old is essential to support the failing heart. inbred control and cardiomyopathic hamsters (treatment duration Table 3 enon and, in this case, the inotropic effect of angiotensin II receptors is associated with a significant reduction in the AT receptors and (3) in this experimental model, the 2 beneficial effects of the AT receptor antagonists already 1 administration of losartan or quinapril. quinapril is related to the blockade of the renin–angiotensin–kinins and, more speciﬁcally, of bradykinin; (2) the role of the renin–angiotensin system as well as to the accumulation of endogenous kinins and, more speciﬁcally, of bradykinin; (2) the role of the renin–angiotensin system in the progression of the cardiomyopathy is more likely that of an adaptive process rather than a causative mechanism involved in the genesis of the pathology.

Hemodynamic parameters were not evaluated during the survival study and, therefore, the contribution of peripheral effects of the drugs on blood pressure and heart rate is unknown. Nevertheless, the very low systolic and diastolic blood pressures of the cardiomyopathic hamsters, as well as the lack of hemodynamic effects of losartan or quinapril that were determined in the a posteriori study, strongly support the conclusion that preload and afterload reductions have played minor roles, if any, in the overall effects of the different treatments. While there is no deﬁnitive explanation for interpreting our results, they do suggest the following hypotheses: (1) the cardioprotective effect of quinapril is related to the blockade of the renin–angiotensin system as well as to the accumulation of endogenous kinins and, more speciﬁcally, of bradykinin; (2) the beneﬁcial effects of the AT₁ receptor antagonists already reported using other experimental models are related to the blockade of AT₁ receptors but also to the activation of AT₂ receptors and (3) in this experimental model, the upregulation of AT₁ receptors is an adaptive phenomenon and, in this case, the inotropic effect of angiotensin II is essential to support the failing heart.

Beneﬁcial effects in experimental models of heart failure have been described with the administration of ACE inhibitors. For example, in myocardial infarcted rats induced by coronary ligation, amelioration of hemodynamic parameters with delapril [26], improvement of cardiac performance with captopril [16], a decrease in ventricular remodeling with enalapril and lisinopril [17,27] and an increase in survival with captopril, enalapril and lisinopril, have been reported [15,27,28]. The increase in the probability of survival that was observed with quinapril was expected since an increase in life expectancy has already been reported in cardiomyopathic hamsters by Haleen et al. [4]. In their study, the treatment was started

<p>| Table 2 | Serum variables measured in inbred control (450-day-old) and cardiomyopathic (end-failure stage, 350-day-old) hamsters treated with placebo, losartan or quinapril |
|---------------------------|-----------------|----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Na⁺ (mmol/l)</th>
<th>K⁺ (mmol/l)</th>
<th>ALT (U/l)</th>
<th>Creatinine (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>144±2⁰</td>
<td>4.7±0.1</td>
<td>74±6</td>
</tr>
<tr>
<td>Losartan (100 mg)</td>
<td>144±2</td>
<td>4.7±0.1</td>
<td>74±6</td>
</tr>
<tr>
<td>Cardiomyopathics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>153±1</td>
<td>5.2±0.3</td>
<td>158±24</td>
</tr>
<tr>
<td>Losartan (30 mg)</td>
<td>154±1</td>
<td>5.9±0.4</td>
<td>174±25</td>
</tr>
<tr>
<td>Losartan (100 mg)</td>
<td>155±1</td>
<td>6.0±0.4</td>
<td>209±27</td>
</tr>
<tr>
<td>Quinapril (100 mg)</td>
<td>146±1⁰</td>
<td>7.3±0.8</td>
<td>129±16</td>
</tr>
</tbody>
</table>

* Mean±SEM, n=15/group.

Table 3 presents the hemodynamic parameters determined in an a posteriori study using 350-day-old anesthetized hamsters following 22±2 days of treatment. As expected, significant decreases were observed in systolic and diastolic blood pressures of cardiomyopathic hamsters compared with the values noted in inbred control animals. Losartan or quinapril had no effect on blood pressure in either groups of hamsters. No differences were noted in heart rate between the inbred control and cardiomyopathic animals and, again, no differences were observed with the administration of losartan or quinapril.

4. Discussion

Our results demonstrate that chronic blockade of AT₁ receptors is associated with a significant reduction in the survival of cardiomyopathic hamsters whereas ACE inhibition improves their life expectancy. Moreover, ventricular hypertrophy is not affected by selective AT₁ antagonism whereas ACE inhibition significantly decreases its development. Compared with the placebo-treated group, the evaluation of the cardiac histological lesions in the four cardiomyopathic groups shows that neither losartan (low- and high-dose) nor quinapril had any effect on the extent and intensity of typical calcification and fibrosis. Although a slight decrease in the number of calcification lesions was observed in animals treated with quinapril, the difference among the groups was not significant. These results indicate that the probability of survival of the cardiomyopathic hamsters obtained following the different interventions on the renin–angiotensin system is independent of the development and/or intensity of the primary cardiac histological lesions. This strongly suggests that the role of the renin–angiotensin system in the progression of the cardiomyopathy is more likely that of an adaptive process rather than a causative mechanism involved in the genesis of the pathology.

Table 3 | Pressor and chronotropic effects of losartan and quinapril in 350-day-old inbred control and cardiomyopathic hamsters (treatment duration=22±2 days) |
<table>
<thead>
<tr>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Systolic pressure (mmHg)</td>
<td>Diastolic pressure (mmHg)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Inbred controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>136±14⁰</td>
<td>98±7</td>
</tr>
<tr>
<td>Losartan (100 mg)</td>
<td>140±11</td>
<td>102±7</td>
</tr>
<tr>
<td>Cardiomyopathics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>54±2⁰</td>
<td>44±3⁰</td>
</tr>
<tr>
<td>Losartan (100 mg)</td>
<td>51±3⁰</td>
<td>41±3⁰</td>
</tr>
<tr>
<td>Quinapril (100 mg)</td>
<td>51±5⁰</td>
<td>39±4⁰</td>
</tr>
</tbody>
</table>

* Mean±SEM, n=7/group.

P≤0.05 compared with the corresponding inbred control group.
in 180-day-old CHF 146 hamsters and quinapril (100 mg/kg/day) increased the median probability of survival of the animals by 33% compared with the untreated group. The major effect on survival occurred during the first 210 days of treatment and, thereafter, the mortality rate of the groups was comparable. In our study, the improvement in life expectancy (+73% in risk of cardiac death, \(P=0.0003\)) related to quinapril was greater and more sustained, with 77% of the hamsters included in this group still alive at the end of the study. This greater effect is certainly related to the fact that quinapril was started in younger animals (50-day-old) before the appearance of cardiac histological lesions and the development of hypertrophy.

In the study by Haleen et al. [4], the improved survival of the cardiomyopathic hamsters associated with quinapril was accompanied by an increase in coronary flow and left ventricular performance, and a decrease in left ventricular dilatation. A cause/effect relationship cannot be established on the basis of our data only. However, the significant decrease in the development of ventricular hypertrophy that was obtained with the administration of quinapril strongly suggests that ACE inhibition increased the life expectancy of the hamsters by decreasing the cardiac remodeling process. This possibility is further strengthened by the fact that the harmful effect observed following AT\(_1\) receptor blockade was related to the absence of effects and even to an increase in the development of cardiac hypertrophy.

The mechanism(s) by which quinapril decreased the development of cardiac hypertrophy is(are) still unclear. One of the most common hypotheses is that the beneficial effects of ACE inhibitors may result not only from a reduced angiotensin II-AT\(_1\) receptor interaction but also from bradykinin potentiation [29]. Studies by Bao et al. [30] as well as by Linz and Scholkens [31] have shown that ventricular hypertrophy induced in spontaneous and aortic-banded hypertensive rats is reduced by treatment with the ACE inhibitor, ramipril. In these models, the regression of cardiac hypertrophy was abrogated by the concomitant administration of HOE-140, a highly potent bradykinin B\(_2\) receptor antagonist. Similar results have also been obtained by McDonald et al. [32] using a model of ventricular remodeling induced by transmyocardial direct current shock in dogs, as well as by Liu et al. [33] using a model of heart failure induced by coronary ligation in rats. Altogether, these results support the hypothesis that the accumulation of endogenous bradykinin, resulting from ACE inhibition, plays a role in the benefit of this therapy in the case of progressive heart failure and, more particularly, in the cardioprotective effect that we and others have observed in the cardiomyopathic hamster.

Treatment with AT\(_1\) antagonists causes elevation of plasma angiotensin II, which may selectively bind to unblocked AT\(_2\) receptors. So far, the roles of AT\(_1\) receptors in physiopathology have not been clarified. However, until now, an inhibitory effect of these receptors on cellular proliferation has been observed using coronary endothelial cells [34], vascular smooth muscle cells [35] as well as cardiomyocytes [36]. It has been suggested by Masaki et al. [37] that AT\(_3\) receptors have an anti-AT\(_1\) effect on cell proliferation and may play a role in development and/or differentiation.

The level of expression of AT\(_3\) receptors is generally low in the adult cardiovascular system and the involvement of these receptors in the progression of heart failure is presently highly speculative. Nevertheless, since their expression is increased in remodeling hearts, such as in cardiac hypertrophy and infarction [38,39], and since the AT\(_2\)-AT\(_1\) ratio is increased in failing human hearts [40,41], these observations suggest that AT\(_2\) receptors might be involved in the effect of angiotensin II. This possibility is supported by the results of Liu et al. [33] who, using a model of cardiac failure induced by coronary ligation in rats, reported that the cardioprotective effect of the AT\(_1\) receptor antagonist L-158,809 was partially abolished by the blockade of AT\(_2\) receptors by PD123319.

We have reported that there are no AT\(_2\) receptors in ventricular membranes of adult cardiomyopathic hamsters (from 30 to 350 days of age) [20]. It is therefore tempting to postulate that the lack of influence of AT\(_1\) receptor blockade on ventricular hypertrophy as well as the negative effect on survival observed in cardiomyopathic hamsters were related, at least in part, to the absence of AT\(_2\) receptor activation and, thereby, of their resulting anti-proliferative effect.

In the last two decades, it has become evident that tissular renin–angiotensin systems exist, including in the myocardium [42]. It is now acknowledged that locally produced angiotensin II may act either in an autocrine or a paracrine fashion to directly modulate cardiac function through a mechanism that is independent of its peripheral action [43,44]. Despite an apparent lack of inotropic response to angiotensin II in the myocardium of the guinea pig and the adult rat, a positive inotropic response to angiotensin II has been demonstrated in cardiac muscle from several species, such as the cat, rabbit, calf and chicken, and in isolated perfused hearts from normal and cardiomyopathic hamsters [45,46]. It has also been reported that angiotensin II exerts positive inotropic effects in human atrial preparations but not in right or left ventricular preparations [47]. When present, physiological studies with specific AT\(_1\) antagonists have proven that the inotropic effect of angiotensin II is mediated by AT\(_1\) receptors [48,49]. The contribution of angiotensin II to the maintenance of the contractile status of a physiological and/or pathological heart is still however not known. Nevertheless, it may be hypothesized that the upregulation of AT\(_1\) receptors observed in the cardiomyopathic hamster heart represents an adaptative mechanism that compensates for the altered contractility and the development of cardiac dysfunction that is secondary to the primary genetic defects. In such a case, the complete inhibition of AT\(_1\)
receptors would be detrimental, especially for the failing heart. This would in turn explain the negative results that we obtained following their chronic blockade.

5. Conclusions

In the cardiomyopathic hamster, losartan did not improve survival compared to placebo and quinapril and, if anything, increased mortality. Major particularities of this experimental model, such as a significant upregulation of cardiac AT₁ receptors and the lack of cardiac AT₂ receptors, may have had a significant impact on our findings and care must be exercised in the interpretation of our results. Nevertheless, they certainly suggest that AT₁ receptor antagonists and ACE inhibitors are not necessarily equivalent or interchangeable in terms of their effects on cardiac hypertrophy and survival in selected progressive heart failure models.

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References


