Effect of long-term therapy with fasidotril, a mixed inhibitor of neprilysin and angiotensin-converting enzyme (ACE), on survival of rats after myocardial infarction


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Abstract

Objective: Two hormonal systems with opposite effects are activated in congestive heart failure: the renin–angiotensin system that promotes vasoconstriction, cardiac hypertrophy and salt retention, and the atrial natriuretic factor (ANF), which has vasorelaxant and natriuretic effects. It could be of therapeutic interest to associate prevention of angiotensin II formation, by inhibition of angiotensin I-converting enzyme (ACE), with potentiation of the ANF effects, by inhibition of neprilysin (NEP).

Methods: The effects of long-term therapy with fasidotril, a mixed NEP/ACE inhibitor, were assessed in rats submitted to coronary artery ligation. Twenty-four hours after ligation, 172 rats were assigned to either placebo or fasidotril therapy (180 mg/kg/day, orally) for 40 weeks. The date of spontaneous death was recorded, myocardial infarct size was determined and rats were classified as having small, moderate or large infarcts.

Results: In rats with moderate infarcts, fasidotril prolonged survival, 50% of the control rats dying during the 40-week observation period compared with 30% of treated rats (P<0.04, log-rank test). In rats with large infarcts, mortality was significantly reduced during the initial 25 weeks of therapy, during which 23.5% of animals died compared to 53.8% in untreated rats (P<0.015). Cardiac hypertrophy was significantly attenuated by fasidotril for the three infarct sizes. Plasma renin activity was not increased by therapy, which presumably reflected the inhibition of renal renin secretion by endogenous ANF. Fasidotril therapy had no significant effects on arterial blood pressure and heart rate.

Conclusion: In addition to its beneficial effects on survival and cardiac hypertrophy, the lack of hypotensive effect of fasidotril is of interest by reducing the risk of renal hypoperfusion and differentiates the mixed inhibitor from selective ACE inhibitors.

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Keywords: Myocardial infarction; Angiotensin-converting enzyme; Neprilysin; Fasidotril; Rat

1. Introduction

Two major hormonal systems with opposing effects are activated in congestive heart failure. The first one, the renin–angiotensin system (RAS), promotes vasoconstriction and salt retention and increases cardiac filling pressure and cardiac workload with resulting deterioration in myocardial function [1]. The importance of this system’s activation is shown by the beneficial effects of inhibitors of angiotensin I-converting enzyme (ACE) in experimental and clinical heart failure [2,3]. The second system, which consists of atrial natriuretic factor (ANF) and related peptides, exerts potentially beneficial diuretic, natriuretic and vasorelaxant effects and inhibits renin and aldosterone secretion [4]. Although the circulating ANF level increases proportionately with the degree of heart failure [5], vasoconstriction and sodium retention predominate, suggesting that ANF levels may not be sufficiently increased to counteract the effects of the RAS.

In spite of the attenuation of renal and hemodynamic responses to exogenous ANF in heart failure [6–8], the
protective role of ANF was demonstrated by various observations. Thus, monoclonal ANF antibodies increased right atrial pressure, left ventricular end-diastolic pressure and systemic vascular resistance in rats that were developing congestive heart failure [9]. Infusion of ANF [10–12], or inhibition of neprilysin (NEP, neutral endopeptidase, EC 3.4.24.11) [13–15], an enzyme responsible for ANF degradation [16,17], improved hemodynamic parameters in patients with congestive heart failure. The potential value of co-inhibition of NEP and ACE has been demonstrated in experimental heart failure. In dogs with pacing-induced heart failure, simultaneous ACE and NEP inhibition produced greater benefits on hemodynamic and renal functions than single inhibition [18,19]. In cardiomyopathic hamsters, the combination of an ACE inhibitor and a NEP inhibitor produced significant decreases in cardiac preload and afterload, whereas each treatment alone had minimal effects [20].

These data have encouraged the development of ‘mixed inhibitors’, i.e. molecules that inhibit both NEP and ACE, and a few studies have reported their potential value in experimental heart failure. A four-week treatment with fasidotril (BP 1.137, previously named alatriopril or aladotril) prevented the development of cardiac hypertrophy and reduced cardiac preload in rats with myocardial infarction [21,22]. Using the same model, acute administration of RB 105 increased natriuresis and augmented urinary excretion of ANF, cGMP and bradykinin [23]. In cardiomyopathic hamsters, acute administration of MS 182657 resulted in hemodynamic effects that were greater than those induced by selective inhibition of NEP or ACE [24]. In the present study, we have tested the effect of long-term treatment with the mixed NEP/ACE inhibitor fasidotril on the survival of rats with myocardial infarction, a model of hypertrophy and overload that rapidly leads to cardiac insufficiency in animals with large myocardial infarcts [25].

2. Methods

2.1. Protocol

Experimental procedures followed in the present study conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1985). Myocardial infarction was induced in 242 male Wistar rats, weighing 250 g, by ligation of the left coronary artery [26] under ether anesthesia. Twenty four hours after ligation, the surviving rats (n=172) were randomized in two groups: a control group (n=86), receiving standard rat chow containing 0.2% sodium (M20 Extralabo, Pierremont, France), and a treated group (n=86), receiving fasidotril [(N(S)-α-(mercaptomethyl)-3,4-(methyl-enedioxy)hydrocinnamoyl)-L-alanine, benzylester, acetate ester, Laboratoire Bioprojet, Paris, France), which was incorporated in the standard chow. Treatment was initiated 24 h after ligation and was continued for 40 weeks. The concentration of fasidotril in the chow was adjusted during the experiment to ensure an average daily intake of 180 mg/kg (range between 160 and 200 mg/kg). An additional group of 19 rats, which were not subjected to an operation, was maintained concurrently with the ligated rats and received standard chow. Drinking water was provided ad libitum in all groups. Each animal was followed until death or for up to 40 weeks. Cages were inspected for dead animals twice daily. Body weight, food and water intake were measured weekly.

2.2. Infarct size and morphological parameters

These parameters were evaluated in animals that died during the experiment or survived after the 40-week observation period. The heart and lungs were removed and weighed. The left and right atria, and the right ventricle were dissected and weighed. The left ventricle (septum+ free wall) was weighed and an estimation of infarct size was made planimetrically as previously described [27]. The left ventricle was opened with an incision along the septum from base to apex. Incisions were made in the left ventricle so that the left ventricular tissue could be pressed flat. The circumferences of the left ventricle and the visualized infarcted area, as judged from both epicardial and endocardial sides, were outlined on a clear plastic sheet. The difference in weight between the two marked areas on the sheet was used to determine the size of the myocardial infarct, which was expressed as a percentage of the left ventricular surface area. Rats were classified as having small (>5%<20%), moderate (>20%<35%), or large (>35%) infarcts.

2.3. Hemodynamic studies

Heart rate and systolic blood pressure were measured noninvasively in conscious unrestrained rats by a standard tail-cuff method (Electrosphygmomanometer PE 300, Narco-Biosystems). The measurements were performed during the 25th week of the experiment in the 19 intact rats and randomly in 27 control ligated rats and in 26 treated ligated rats.

Another invasive hemodynamic study was performed in rats that survived at the end of the experiment. The rats were anesthetized with halothane and cannulated with catheters filled with heparinized saline. A polyethylene catheter (PE10, Merck-Biotrol) was placed in the right femoral artery for measurement of mean arterial blood pressure (MABP) and heart rate. A polyethylene catheter that consisted of PE10 tubing welded to PE50 tubing was introduced into the left ventricle via the right carotid artery, to measure left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP) and the
maximum rate of rise of left ventricular pressure (+dP/dt). The catheters were exteriorized at the nape of the neck and connected to Gould-Statham pressure transducers and to a Gould 8000S polygraph. The rats were allowed to recover unrestrained in a cage for at least 4 h before initiating measurements.

2.4. Plasma enzyme activities

Plasma renin, ACE and NEP activities were measured in rats with moderate infarcts that survived after the 40-week observation period. Blood was collected after decapitation, 12 h after fasidotril withdrawal. For measurement of renin activity, blood was collected in EDTA (1.5 mg/ml) and enzyme activity was evaluated in the presence of an excess of substrate [28]. Angiotensin I was radioimmunoassayed (Amersham) after incubation at 37°C for 1 h in the presence of an excess of plasma from nephrectomized rats. For measurement of ACE and NEP activities, blood was collected into heparinized tubes. Plasma ACE activity was evaluated using 0.2 mM o-aminobenzoylglycyl-p-nitrophenylalanylproline as the substrate [29]. Plasma NEP activity was determined by a two-step fluorimetric assay with 25 µM succinyl-Ala-Ala-Phe-amidomethylcoumarin as the substrate [30]. Blanks were obtained by adding 1 µM thiorphan to the substrate solution in parallel incubations.

2.5. Statistical analysis

The data are given as means±SEM, and comparisons were made using one-way analysis of variance followed by Newman-Keuls test. Differences in distribution of infarcts and in mortality rates were investigated by the chi-squared test. Comparison of survival curves was performed by the log-rank test. Differences were considered to be statistically significant when P<0.05.

3. Results

3.1. Evolution of body weights, food and water intakes

The evolution of body weights during the observation period was similar in the three experimental groups (unligated, ligated control and ligated fasidotril) except during the first week after operation. At this time, a decrease in body weight (about −20 g) was observed in ligated rats compared with unligated rats. Afterwards, the evolution of body weight was similar in the three groups and, at the end of the study, the body weight of the surviving animals was 806±15 g in the unligated group, 807±16 g in the ligated control group, and 792±14 g in the ligated fasidotril group. Food intake (26–29 g chow per day per rat) was relatively constant during the experiment, except for ligated rats during the first week after operation, during which food intake was reduced by 20–30%. Water consumption was initially augmented in the fasidotril-treated group. The effect was maximal during the first week of treatment. After seven days of treatment, water consumption reached 34.9±1.0 ml/day/rat in the ligated control group vs. 41.5±1.0 ml/day/rat in the fasidotril-treated group (+19%, P<0.05). Afterwards, the difference lessened and was no longer significant during and after the third week of treatment.

3.2. Infarct size and survival

The distribution of myocardial infarct sizes was not significantly different among the untreated and the treated groups (chi-squared test, P=0.21) and infarct sizes did not differ significantly within the two groups for any of the infarct size classifications (Table 1).

None of the unligated rats (n=19) died during the 40-week observation period. The mortality of rats with small infarcts was very low in both groups. Only one rat from the untreated group (n=12) died on day 183 and one rat from the treated group (n=6) died on day 242.

The survival curves for rats with moderate infarcts are shown in Fig. 1A. In the untreated group (n=48), 24 rats died during the observation period (50% mortality), whereas in the treated group (n=46), only 14 rats died (30% mortality). Comparison of the survival curves by the log-rank test indicated a significant effect of the treatment (P=0.04). With the 50% survival rate being undefined in the treated group (greater than the 280-day observation period), the calculated 75% survival rate reached 175 days in the untreated group vs. 265 days in the treated group.

In rats with large infarcts (Fig. 1B), mortality was very high in both groups, reaching 96% in the untreated group and 94% in the treated group. There was no significant difference in the 50% median survival, which was 171 days in the untreated group (confidence interval 95%: 116–217 days) and 214 days in the treated group (confidence interval 95%: 194–236 days). However, during the first months of treatment, the rate of death appeared to be greater in the untreated group. So, during the initial 25 weeks of therapy, 14 of 26 rats died in the untreated group.

<table>
<thead>
<tr>
<th>Infarct size classifications</th>
<th>Number of rats</th>
<th>Infarct size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small infarcts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated group</td>
<td>12</td>
<td>15.9±0.9</td>
</tr>
<tr>
<td>Fasidotril group</td>
<td>6</td>
<td>13.9±1.8</td>
</tr>
<tr>
<td>Moderate infarcts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated group</td>
<td>48</td>
<td>29.1±0.5</td>
</tr>
<tr>
<td>Fasidotril group</td>
<td>46</td>
<td>28.0±0.6</td>
</tr>
<tr>
<td>Large infarcts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated group</td>
<td>26</td>
<td>40.6±1.0</td>
</tr>
<tr>
<td>Fasidotril group</td>
<td>34</td>
<td>40.1±0.8</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM.
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(53.8% mortality) versus eight of 34 rats (23.5% mortality) in the treated group (P=0.015, chi-squared test). Fig. 1C shows the survival curves, including moderate+large infarcts. Fasidotril had no significant effect on mortality at 40 weeks but it reduced the mortality rate till day 212 (P=0.04, chi-squared test).

Rats which died during the experiment showed evidence of congestive heart failure, as indicated by fluid accumulation in the thoracic cavity and by a marked increase in the lung weight. So, the relative lung weight (lung weight/body weight) was equal to 2.33±0.09 mg/g in unligated rats. In rats with large infarcts which died during the experiment, it reached 7.11±0.43 mg/g (n=25) in the untreated group and 6.87±0.30 mg/g (n=32) in the treated group (N.S. versus untreated group). In rats with moderate infarcts, the values were 6.54±0.61 mg/g (n=24) in the untreated group and 6.15±0.48 mg/g (n=14) in the treated group (N.S. versus untreated group).

3.3. Cardiac hypertrophy

Cardiac hypertrophy was evaluated by cumulating rats which died and survived during the observation period (Fig. 2). The data were classified according to infarct sizes and the tissue weights were normalized to body weights and expressed as relative weights. In rats with small or moderate infarcts, the body weights were not significantly different in the three experimental groups, but rats with large infarcts, which had a shortened survival time, exhibited significant reductions in body weight compared to unligated rats (Fig. 2).

Myocardial infarction caused significant myocardial hypertrophy, which increased with infarct size. So, compared to unligated rats, the relative weight of the whole heart in untreated ligated rats was increased by +18, +43 and +102% in rats with small, moderate and large infarcts, respectively. Hypertrophy was evident in both ventricular and atrial chambers and was particularly accentuated in the atria and the right ventricle (Fig. 2). In the left ventricle, the ligation-induced loss of myocardial tissue was compensated for by reactive hypertrophy, resulting finally in significant but moderate hypertrophy. The increase in relative weight, for the three infarct sizes, reached +17, +21 and +33% for the entire left ventricle and +18, +51 and +89% for the non-infarcted septum.

Fasidotril treatment resulted in significant attenuation of cardiac hypertrophy (Fig. 2). For the whole heart, the increase in relative weight was completely prevented in rats with small infarcts and significantly attenuated in rats with moderate infarcts (+25% vs. +43% in untreated rats) and large infarcts (+70% vs. +102% in untreated rats). Attenuation of hypertrophy was seen to various extents in the different heart chambers. For the left ventricle, significant attenuation of hypertrophy was seen for the three infarct sizes. For the right ventricle and right atrium, the effect was significant only in rats with large infarcts, which
Fig. 2. Body weights and relative weights of cardiac structures (tissue weight/body weight) in unligated (U) or ligated rats with small (S), moderate (M) or large (L) infarcts. Rats were followed until death or up to 40 weeks and the data represent the cumulated values obtained for rats that died and survived. Values are expressed as mean±SEM. n=number of rats. *P<0.05 versus unligated rats. P<0.05 between untreated and fasidotril-treated rats.

exhibited marked hypertrophy of these structures. For the left atrium, the antihypertrophic effect of fasidotril was significant only in rats with small infarcts (Fig. 2). However, since more rats of the untreated group died before the end of the observation period compared with the treated group, the extent of cardiac hypertrophy in the untreated group is probably underestimated.

Cardiac hypertrophy was also evaluated in moderate-sized infarct groups that had enough survivors at the end of the study. The results indicated that these surviving rats exhibited a weak but significant cardiac hypertrophy. So, comparison between unligated rats (n=19) and untreated ligated rats (n=24) showed that the relative ventricular weight was increased from 1.24±0.03 to 1.41±0.03 mg/g for the left ventricle (P<0.05), and from 0.31±0.01 to 0.42±0.02 mg/g for the right ventricle (P<0.05). In rats receiving fasidotril (n=32), there was a significant diminution of the left ventricular weight (1.31±0.02 versus 1.41±0.03 mg/g in the untreated group, P<0.05) but the right ventricular weight was not significantly altered by treatment (0.40±0.02 versus 0.42±0.02 mg/g in untreated rats).
SBP, systolic blood pressure; MABP, mean arterial blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure.

Measurements were performed 25 or 40 weeks after initiation of fasidotril therapy.

Values are expressed as mean±SEM. n=number of rats.
Measurements were performed 25 or 40 weeks after initiation of fasidotril therapy.
SBP, systolic blood pressure; MABP, mean arterial blood pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt, maximum of rise of left ventricular pressure.

3.4. Hemodynamic parameters

Systolic blood pressure and heart rate were measured during the 25th week of the study in 19 unligated rats and randomly in 27 untreated and 26 treated ligated rats. Subsequent determination of the infarct sizes indicated that the untreated ligated group contained six rats with small infarcts, 17 rats with moderate infarcts and four rats with large infarcts, whereas the treated ligated group contained two rats with small infarcts, 16 rats with moderate infarcts and eight rats with large infarcts. Data were analyzed only in rats with moderate infarcts. As indicated in Table 2, systolic blood pressure and heart rate were not modified by fasidotril treatment. However, systolic blood pressure was significantly lowered (about −10 mmHg) in the ligated groups compared to the unligated group.

At the end of the study, measurements were performed in the unligated rats and in the surviving rats with moderate infarcts. Because of technical problems associated with intracardiac catheterization, successful measurements were obtained only for 13 unligated rats, 12 untreated ligated rats and 23 treated ligated rats (Table 2). Infarction resulted in significant reductions in mean arterial blood pressure (MABP) and LVSP (−10 to −15 mmHg) and of +dP/dt but had no significant effect on LVEDP and on heart rate. Fasidotril treatment had no effect on LVEDP, +dP/dt and heart rate and led to slight (non-significant) diminutions of MABP and LVSP (−5 mmHg). The lack of augmentation of LVEDP in the surviving animals with moderate infarcts indicated that heart failure was well compensated for in these rats, as shown by the weak cardiac hypertrophy (see above) and by a weak increase in the lung weight, an indicator of congestive heart failure. So, the relative weight of the lungs was equal to 2.33±0.09 mg/g in the unligated rats and to 2.88±0.19 mg/g in the infarcted rats (P<0.05). In comparison, this value reached 6.87±0.30 mg/g in rats with moderate infarcts that died during the experiment.

3.5. Plasma enzyme activities

Measurements were made on rats with moderate infarcts that survived at the end of the study. Blood was collected about 12 h after withdrawal of fasidotril. As shown in Table 3, plasma ACE and NEP activities were similar in unligated and in untreated ligated rats. Fasidotril treatment resulted in a decrease in NEP activity (−75%) and in a two-fold increase in ACE activity. Plasma renin activity was similar in the three groups.

Table 2
Effect of fasidotril treatment on hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>After 25 weeks</th>
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<tbody>
<tr>
<td></td>
<td>Unligated (n=19)</td>
<td>Untreated ligated moderate infarcts (n=17)</td>
<td>Fasidotril ligated moderate infarcts (n=16)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>128±2</td>
<td>116±3**</td>
<td>118±3*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>327±4</td>
<td>332±5</td>
<td>332±6</td>
</tr>
<tr>
<td>After 40 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unligated (n=15)</td>
<td>Untreated ligated moderate infarcts (n=12)</td>
<td>Fasidotril ligated moderate infarcts (n=23)</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>118±3</td>
<td>109±2*</td>
<td>103±2*</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>145±3</td>
<td>134±3*</td>
<td>129±3*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.7±0.9</td>
<td>5.2±1.0</td>
<td>5.3±1.0</td>
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<tr>
<td>+dP/dt (mmHg/s)</td>
<td>4654±278</td>
<td>3817±220**</td>
<td>3604±179**</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>347±12</td>
<td>357±8</td>
<td>352±9</td>
</tr>
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</table>

Values are expressed as mean±SEM. n=number of rats.
Measurements were performed 25 or 40 weeks after initiation of fasidotril therapy.

Table 3
Effect of fasidotril treatment on plasma enzyme activities

<table>
<thead>
<tr>
<th></th>
<th>Unligated (n=17)</th>
<th>Untreated ligated (n=17)</th>
<th>Fasidotril ligated (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE (nmol/ml/min)</td>
<td>11.3±0.5</td>
<td>12.2±0.4</td>
<td>23.0±0.5**</td>
</tr>
<tr>
<td>NEP (pmol/ml/min)</td>
<td>44.0±8.9</td>
<td>42.8±5.7</td>
<td>10.7±1.0**</td>
</tr>
<tr>
<td>Renin (ng angiotensin I/ml/h)</td>
<td>30.5±3.4</td>
<td>36.1±7.4</td>
<td>32.1±3.5</td>
</tr>
</tbody>
</table>

Measurements were performed 40 weeks after initiation of fasidotril therapy in rats with moderate infarcts.
Blood was collected 12 h after fasidotril withdrawal.
Values are expressed as mean±SEM. n=number of rats.

*P<0.05 versus unligated rats. **P<0.05 between untreated ligated and fasidotril ligated rats.
4. Discussion

Some properties of the mixed NEP/ACE inhibitor fasidotril (previously named alatriopril or aladotril) have been described [31]. This compound inhibited both NEP and ACE in vitro with similar nanomolar potencies and exerted typical actions of ACE inhibitors and of NEP inhibitors, such as inhibition of angiotensin I-induced hypertension, protection of ANF and enhancement of diuresis, natriuresis and cGMP urinary excretion in rats submitted to volume expansion [31]. The dose of fasidotril used in this study (180 mg/kg/day, orally) was selected from previous data showing that, in the rat, this dosage inhibited the pressor response to angiotensin I, potentiated the depressor effect of bradykinin and exerted marked inhibition of plasma ACE and NEP activities [21]. So, a four-week treatment of infarcted rats with fasidotril (100 mg/kg, orally, twice daily) resulted in 67% inhibition of plasma ACE activity and in 77% inhibition of plasma NEP activity, blood being collected 2 h after fasidotril administration.

Long-term therapy with fasidotril did not modify the evolution of body weight and food intake in comparison with untreated rats. The initial increase in water consumption that was observed after initiation of fasidotril therapy could be the consequence of ACE inhibition. A similar dipsogenic effect has been reported after administration of ACE inhibitors such as captoril [32] or idapril [33]. A tentative explanation is an increased circulating level of angiotensin I after ACE blockade, diffusion of angiotensin I to brain sites that are devoid of a blood–brain barrier, conversion to angiotensin II, and binding to angiotensin II receptors mediating excessive thirst.

The present study demonstrates for the first time that long-term therapy with a mixed NEP/ACE inhibitor prolonged survival of rats submitted to myocardial infarction. Significant improvement in survival was observed in rats with moderate infarcts. In rats with large infarcts, fasidotril treatment had no beneficial effect on the 40-week mortality rate but led to an initial improvement of survival (five–six weeks) with captopril and hydralazine, which was started 21 days after ligation. By comparing the effects of chronic treatments with untreated rats. The initial increase in water consumption that was observed after initiation of fasidotril therapy could be the consequence of ACE inhibition. A similar dipsogenic effect has been reported after administration of ACE inhibitors such as captoril [32] or idapril [33]. A tentative explanation is an increased circulating level of angiotensin I after ACE blockade, diffusion of angiotensin I to brain sites that are devoid of a blood–brain barrier, conversion to angiotensin II, and binding to angiotensin II receptors mediating excessive thirst.

The present study demonstrates for the first time that long-term therapy with a mixed NEP/ACE inhibitor prolonged survival of rats submitted to myocardial infarction. Significant improvement in survival was observed in rats with moderate infarcts. In rats with large infarcts, fasidotril treatment had no beneficial effect on the 40-week mortality rate but led to an initial improvement of survival during the first months of therapy. Although our experimental design did not include animal groups that were treated only with ACE inhibitor or NEP inhibitor exclusively, our results can be compared with those of previous studies that have evaluated the effect of ACE inhibitors on the survival of infarcted rats. It was reported that long-term therapy with captopril [34], enalapril [35] or lisinopril [36] enhanced survival in rats with moderate infarcts but not in rats with large infarcts [34,36]. Comparison of our results with those reported by Pfeffer et al. [34] using captopril (2 g/l of drinking water) indicated that the difference in mortality between untreated and treated rats with moderate infarcts was 27% after 52 weeks of captopril treatment and 20% after 40 weeks of fasidotril treatment. In rats with large infarcts, Pfeffer’s study [34] showed a trend towards improved survival till about 122 days, whereas in our study, fasidotril treatment resulted in a significant improvement in survival until 175 days ($P=0.015$).

It is worth noting that, in the studies using ACE inhibitors, initiation of therapy was made one week [35,36] or two weeks [34] after coronary artery ligation, whereas in our study, treatment was started 24 h after ligation, i.e. at a time when infarct scar was not achieved, completion of the healing process requiring about 21 days in the rat [37]. The possibility exists that starting treatment early after ligation could result in greater benefit than that of treatment initiated later, by reducing infarct expansion and exerting earlier effects on the non-infarcted myocardium. However, in this model of infarcted rats, the benefits of early treatment with ACE inhibitors has not been demonstrated. So, initiation of captopril therapy 2 h [38] or 24 h [25] after ligation did not appear to produce greater effects on hemodynamics and cardiac hypertrophy than treatment that was started 21 days after ligation.

Numerous studies have reported that administration of ACE inhibitors for some weeks or months prevented the cardiac hypertrophy and ventricular remodeling that develops after myocardial infarction and the beneficial effects have been attributed to local and systemic effects. Treatment of heart failure with ACE inhibitors leads to reduction of both cardiac preload and afterload and the combination of these two hemodynamic effects seems to be required for reduction of cardiac hypertrophy and long-term mortality. All of the studies that demonstrated an improvement in long-term survival in rats with myocardial infarction [34–36] have been performed using hypotensive doses of ACE inhibitors. Using two doses of lisinopril, a low dose without effect on arterial blood pressure, although exerting significant inhibition of circulating and pulmonary ACE, and a high dose that caused a sustained decrease in blood pressure, Wollert et al. [36] have demonstrated that only the high dosage improved survival and reduced cardiac hypertrophy. However, afterload reduction alone did not affect protection in this model of infarction. By comparing the effects of chronic treatments (five–six weeks) with captopril and hydralazine, which produced similar reductions in blood pressure, Raya et al. [39] reported that only captopril prevented cardiac hypertrophy and left ventricular dilatation.

Local systems seem to have important roles in the regulation of cardiovascular functions and they may be influenced by both ACE and NEP inhibition [40]. Activation of the tissue RAS in the heart has been described after coronary ligation in the rat [41], which could represent a target for ACE inhibitors. Angiotensin II, generated locally, may be a mediator of ventricular hypertrophy via its stimulating effect on myocyte growth and collagen synthesis [42,43]. NEP is expressed in heart [44] and there is evidence of ANF having inhibitory properties on cell growth [45]. It has been reported that chronic treatment with a NEP inhibitor, SCH 34826, in spontaneously hypertensive rats, reduced cardiac mass and collagen...
synthesis despite the lack of antihypertensive activity [46].
In rats with myocardial infarction, a four-week treatment
with the NEP inhibitor SQ 28603 produced a slight but
significant reduction in cardiac hypertrophy without lowering
arterial blood pressure [47].
In heart failure, enhancement of ANF activity after inhibition
of NEP may counteract the growth-promoting effect of angiotensin II,
leading to an additional reduction in cardiac hypertrophy. By comparing the effects of
captopril and fasidotril, at doses that exerted similar
inhibition of plasma ACE, in infarcted rats, we have reported [21] that fasidotril showed a greater efficacy than
captopril in reducing cardiac hypertrophy. Reduction of
cardiac hypertrophy was also evidenced in the present
study after long-term therapy with fasidotril. Only a few studies
have evaluated the effect of long-term therapy with
ACE inhibitors on cardiac hypertrophy in infarcted rats.
Taking into account the number of animals that survived
and died during the study, a significant reduction in
ventricular hypertrophy was reported with lisinopril [36]

Measurements of plasma enzyme activities in rats with
moderate infarcts that survived for 40 weeks after coronary
ligation indicated that fasidotril treatment did not change
renin activity, reduced NEP activity and augmented ACE
activity, the measurements being performed 12 h after
withdrawal of fasidotril. These opposite effects towards
NEP and ACE may appear surprising for a drug that, in
vitro, possesses similar potencies against both enzymes
[31]. Increase in ACE activity probably reflects induction
of the enzyme in response to its inhibition by fasidotril.
Angiotensin II regulates pulmonary ACE mRNA expres-
sion via a negative feedback mechanism [48] and induction
of pulmonary ACE has been demonstrated after adminis-
tration of ACE inhibitors [49]. In rat lung, ACE is
predominantly expressed in vascular endothelial cells and
probably constitutes the main source of circulating ACE
[50]. Increases in plasma ACE activity have been reported
during captopril treatment [51–54], the increase being accentuated after treatment of samples with chloramidine T,
30-fold in plasma renin activity. In contrast, ANF inhibits
renin secretion [57] and ANF infusion lowered renin
secretion in patients with heart failure [58,59]. Conversely,
in dogs with pacing-induced heart failure, blockade of
biologically active ANF receptors resulted in an increase
in plasma renin activity [60], demonstrating the regulatory
role of ANF on renin secretion. However, in dogs with
advanced heart failure, there was an attenuation of the
inhibitory effect of ANF on renin secretion [61], possibly
due to the down-regulation of ANF receptors. Progressive
attenuation of this ANF effect with progression of heart
failure might explain why the beneficial effect of fasidotril
on survival of our rats with large infarcts was not sustained
but was observed only during a limited period.

We have previously reported [22] that a four-week
treatment with fasidotril (200 mg/kg/day, orally) resulted
in reduction of LVHEDP in rats with moderate or large
infarcts but had no effect on arterial blood pressure,
whereas captopril therapy (20 mg/kg/day, orally) led to
diminution (−15 mmHg) of mean arterial blood pressure.
The lack of an hypotensive effect of fasidotril is confirmed
in the present study in which blood pressure measurements
were made after 25 and 40 weeks of therapy. This lack of
depressor effect is surprising for a drug that displays high
inhibitory activity towards ACE. By evaluating the effect
of another mixed inhibitor (RB 105) on the blood pressure
of infarcted rats, Gonzalez et al. [23] reported that the drug
erected a minimal hypotensive effect (−5 mmHg). A
possible explanation for the lack of an hypotensive effect
of fasidotril is that simultaneous NEP/ACE inhibition
results in a lesser activation of RAS than that of ACE
inhibition alone. The hypotensive effect of ACE inhibitors
in rats with myocardial infarction demonstrates the impor-
tance of the RAS in maintaining blood pressure in this
pathological situation. Hypotension and reduction of renal
perfusion pressure cause additional activation of the sys-
tem, leading to a vicious circle, which may be prevented
by concomitant NEP inhibition, as evidenced by the lack of
augmentation of renin secretion.

Interactions between ANF and RAS do not rule out
other mechanisms, due to the lack of specificity of NEP
and ACE. Both enzymes are implicated in the degradation
of bradykinin, which can contribute to the cardioprotective
effect of ACE inhibitors, an effect that may be potentiated
by simultaneous NEP inhibition. In cardiomyopathic ham-
sters, inhibition of bradykinin B2 receptors blunted the
decrease in left ventricular end-diastolic pressure induced
by dual NEP/ACE inhibition, suggesting that bradykinin
potentiation contributes to the hemodynamic effects of
NEP/ACE inhibition [62]. NEP can also hydrolyse sub-
strates other than ANF and bradykinin, including angioten-
sin I and II [63] and endothelin [64], that can contribute to
the effects of mixed NEP/ACE inhibitors.

In heart failure, a treatment that does not lower blood
pressure is potentially of interest, by reducing the risk of
hypoperfusion. In addition, because of the high sensitivity
of the natriuretic effect of ANF to changes in blood pressure [65], the maintenance of renal perfusion pressure is favourable to the full expression of the renal effects of ANF. The blunted renal responses to ANF that have been reported in severe congestive heart failure [6,8] are probably partly mediated by an excessively low blood pressure. Renal hypoperfusion may cause functional renal insufficiency by reducing the glomerular filtration rate in patients in which glomerular filtration is obviously angiotensin II-dependent [66]. Detrimental effects of ACE inhibitors, caused by a reduction of the coronary perfusion pressure, have also been reported in patients with heart failure and angina pectoris [67]. In terms of safety, the potential dangers of the use of ACE inhibitors in the course of acute myocardial infarction is an unpredictable reduction of blood pressure and initiation of therapy should be carefully titrated to avoid excessive hypotension [68,69]. In this regard, the results of the present study, awaiting the results of clinical studies, show the potential value of the mixed NEP/ACE inhibitor fasidotril in heart failure treatment.

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References


