Long-Term Survival and Hemodynamics After Endothelin-A Receptor Antagonism and Angiotensin-Converting Enzyme Inhibition in Rats With Chronic Heart Failure

Monotherapy Versus Combination Therapy

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Background—In patients with congestive heart failure (CHF) receiving ACE inhibitors, acute administration of selective endothelin (ET) antagonists additionally improves systemic and cardiac hemodynamics. We investigated, in a rat model of CHF, whether such acute synergistic effects are sustained and accompanied, in the long term, by an additional limitation of left ventricular remodeling or an increase in survival.

Methods and Results—Rats were subjected to coronary artery ligation and treated for 3 or 9 months with vehicle or with the ACE inhibitor trandolapril (Tr) (0.3 mg/kg per day), the ETα antagonist LU 135252 (LU, 30 mg/kg per day), or their combination starting 7 days after ligation. After 3 months, the combination decreased LV systolic- and end-diastolic pressures (−32% and −80%, respectively) more markedly than Tr (−21% and −61%, respectively) or LU alone (−14% and −48%, respectively). Echocardiographic studies revealed that all treatments limited LV dilatation and increased LV fractional shortening and cardiac index. All treatments equally reduced left ventricular collagen density, whereas only Tr or the combination reduced LV weight. Finally, although LU did not modify long-term survival, Tr and the combination of Tr with LU induced a similar improvement of survival.

Conclusions—In this rat model, long-term combined administration of an ETα antagonist and an ACE inhibitor induces additional effects in terms of systemic and cardiac hemodynamics; however, this is not associated with an additional increase in long-term survival. (Circulation. 2002;106;1159-1164.)

Key Words: endothelin ■ hemodynamics ■ heart failure ■ inhibitors, angiotensin-converting enzyme

In chronic heart failure (CHF), plasma levels of endothelin (ET) are increased and are of prognostic value, suggesting a pivotal role of the ET system in the pathophysiology of CHF. In humans and animal models of CHF, acute administration of either mixed ETα-ETβ or selective ETα antagonists exerts beneficial effects in terms of systemic and cardiac hemodynamics, whereas chronic administration also prevents left ventricular remodeling and myocardial collagen accumulation. Concerning long-term survival, contradictory results have been reported. Although mixed ETα-ETβ antagonists significantly improve survival, ETα antagonists either improve or have no effect on long-term survival in experimental CHF.

Although ACE inhibitors decrease cardiac preload and afterload, reduce the progression of cardiac remodeling, and improve long-term survival, the long-term outcome of patients with CHF remains poor. This suggests that other mechanisms remain or become activated after ACE inhibition. For example, in animal and humans with CHF under ACE inhibitor therapy, acute or semichronic administration of ET antagonists provokes an additional decrease in cardiac preload and afterload, illustrating that the ET system remains activated during ACE inhibitor treatment. However, whether the additional hemodynamic effects of combined ET receptor blockade/ACE inhibition are sustained or result in a more marked effect on ventricular remodeling and survival compared with ACE inhibition is unknown.

Thus, the goals of the study were to evaluate in a rat model of CHF induced by coronary artery ligation whether a combined administration of an ETα antagonist and an ACE inhibitor induces long-term additional effects on hemodynamics, left ventricular remodeling, and survival.

Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996).
Animals
Myocardial infarction was produced in 11-week-old male Wistar rats by left coronary artery ligation, using the technique described by Pfeffer et al.16 and modified in our laboratory.9 In brief, animals were anesthetized (sodium methohexital: 50 mg/kg, IP), intubated, and ventilated. After a left thoracotomy, myocardial infarction was induced by ligation of the proximal left coronary artery. Fifteen minutes after occlusion, the chest was closed and the animals were allowed to recover from anesthesia. With this procedure, mortality rate is reduced from 50% to 20%, thus of the 650 rats that underwent left coronary artery ligation, 130 died before inclusion at the seventh postoperative day.

Treatments and Study Design
Seven days after ligation, infarcted rats were randomized to receive either placebo (untreated), the ET<sub>A</sub> receptor LU 135252 (LU; 30 mg/kg per day<sup>17</sup>), the ACE inhibitor trandolapril (Tr; 0.3 mg/kg per day<sup>17</sup>), or their combination. All treatments were provided by Knoll AG (Ludwigshafen, Germany) and given as food admix. Treatments were started 7 days after ligation, in agreement with previous studies, to avoid interference with the healing process or the early distention of the infarct zone. Rats were weighed every week and their food intake was measured to adjust the dosage of the drug concentrations in the chow.

In the first protocol, evaluating whether selective ET<sub>A</sub> receptor blockade improves long-term survival in CHF, infarcted rats were either untreated (n=50) or treated with LU 135252 (n=50). Twelve sham-operated rats were used as controls. In a second protocol evaluating whether selective ET<sub>A</sub> receptor antagonism provides an additional benefit over ACE inhibition treatment, infarcted rats were either untreated (n=120) or treated with the ACE inhibitor trandolapril (n=120) or the combination Tr-LU. Eleven sham-operated rats were used as controls.

Although we assessed the effects of treatments on cardiac hemodynamics and cardiac structural changes after 9 months of treatment, interpretation of these results is rendered difficult by the fact that mortality may cause a selection of animals with only moderate cardiac dysfunction, especially in the untreated group. Thus, to avoid this experimental bias, we performed an additional series of rats that were sacrificed after 3 months of treatment, ie, before any significant difference in mortality had occurred. The 3-month study consisted of one single protocol. Because this study did not include assessment of survival, the number of animals in each group was lower than that in the 9-month studies. Thus, 60 infarcted rats were used. They were either untreated (n=12) or treated with Tr (n=16), LU (n=16), or LU+Tr (n=16). Ten sham-operated rats were used as controls.

Hemodynamic Measurements
Systolic blood pressure (plethysmography) and heart rate were determined in conscious rats before treatment, ie, 1 week after the coronary artery ligation, and again after 1, 3, 6, and 9 months of treatment.

Cardiac hemodynamics was assessed at the end of the 3- and 9-month treatment periods. In brief, the rats were anesthetized (pentobarbital; 50 mg/kg, IP) and the right carotid artery was cannulated with a micromanometer-tipped catheter (SPR 407, Millar Instruments) for recording of arterial pressure. The catheter was then advanced into the left ventricle (LV) for recording of LV pressure and its maximal rate of rise (dP/dt<sub,max</sub>) and decrease (dP/dt<sub,min</sub>).

Echocardiographic Studies
Transthoracic Doppler echocardiographic studies were performed in rats from the 3-month protocol just before the start of the treatment, ie, 7 days after the surgical procedure and after 1 and 3 months of treatment in 3-month treatment. For this purpose, rats were anesthetized (sodium methohexital: 50 mg/kg, IP), the chest was shaved, and echocardiograms were obtained with an echocardiographic system equipped with a 8-MHz transducer (ATL HDI 5000), as described previously.9,15 A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscle, to record M-mode tracings. End-diastolic and end-systolic wall posterior thickness and LV diameters were measured according to the American Society of Echocardiography leading-edge method from at least 3 consecutive cardiac cycles.20

LV outflow velocity was measured by pulsed-wave Doppler, and cardiac index was calculated as CI=[aortic VTI×(π×[LV outflow diameter/2]<sup>2</sup>×heart rate)/body weight, where VTI is velocity-time integral.

Cardiac Morphometry
Morphometric analysis was performed as described previously.21 The atri and left and right ventricles were weighed separately, and the left ventricle was immersed in Bouin fixative solution. After fixation, the heart was cut perpendicular to the apex to base axis into 3 sections of approximately identical thickness. Sections were dehydrated and embedded in paraffin. From these sections, 5-μm-thick histologic slices were obtained and were stained with Sirius red.

For the measurement of infarct size, slices were placed under a video microscope with a 20-fold enlargement lens. The endocardial and epicardial circumferences of the infarcted tissue and of the left ventricle were determined using an image analysis software (Cyberview, Cervus International). Infarct size was calculated as (endocardial+epicardial circumference of the infarcted tissue)/(endocardial+epicardial circumference of the left ventricle) and expressed as a percentage.

For the measurement of cardiac collagen density, slides stained with Sirius red were enlarged 500 times using a microscope connected to the same image analysis system. Collagen density was then calculated as the surface occupied by collagen divided by the surface of the image. Perivascular collagen was excluded from this measurement.

Statistical Analysis
All results, except survival, are given as mean±SEM. Because the values obtained for the untreated CHF groups in both 9-month survival studies were identical, data concerning systemic and cardiac hemodynamics and cardiac morphology were pooled. Comparison of survival in untreated and treated CHF rats was performed according to Kaplan-Meier. For all other parameters, differences between values obtained at each time point were evaluated by ANOVA, followed, if ANOVA revealed significant differences, by a Tukey test for multiple comparisons. Differences were considered significant at the level of P<0.05.

Results
Infarct Size
In the 3-month protocol, infarct size of the untreated or rats treated with LU, Tr, or LU+Tr was 44±2%, 46±2%, 43±2%, and 44±3%, respectively, whereas in the 9-month protocol infarct size was 45±2%, 46±2%, 49±2%, and 48±2%, respectively.

Survival
During the 9-month study period, none of the sham animals died. Figure 1 illustrates the survival curves in CHF rats in the two survival studies. In both control groups, survival at 9 months (23% and 25% in studies 1 and 2, respectively) and median survival time (158 and 157 days, respectively) were similar.

In the first survival study, mortality rate of animals treated with LU at the dose of 30 mg/kg per day was similar to that of untreated animals. Indeed, 9-month survival and median survival time in the LU treated group (17% and 141 days) were similar to those of untreated CHF rats. In the second survival study, rate of death in the Tr-treated CHF group was
significantly lower that that of the untreated CHF group. Nine-month survival rate after 9 months and median survival time in the Tr group (56% and 215 days, respectively) were superior to that of untreated CHF animals. Administration of LU + Tr improved survival to the same extent as Tr alone. Indeed, 9-month survival and median survival time in the LU + Tr CHF group were 54% and 208 days, respectively.

Systemic Hemodynamics in Conscious Rats
After 1 month of treatment, LU or Tr administered alone induced a small but significant decrease of blood pressure, which lasted throughout the 9-month treatment period, whereas heart rate was not modified (Figure 2). Concomitant administration of LU and Tr induced a significantly more marked reduction of blood pressure than LU or Tr alone, and this persisted throughout the 9-month treatment period. Moreover, concomitant administration of LU and Tr moderately reduced heart rate compared with untreated animals (Figure 2).

Left Ventricular Dilatation and Function
LU and Tr significantly limited the increase of left ventricular diameter observed over time during the 3-month treatment period. The limitation of left ventricular dilatation was accompanied by a significant increase in left ventricular fractional shortening (Figure 3). LU + Tr reduced left ventricular dilatation and increased fractional shortening to the same extent as LU or Tr alone.

Cardiac Index and Stroke Volume
When compared with untreated CHF animals, LU and Tr increased cardiac index and stroke volume at all time points during the 3-month treatment period. Cardiac output and stroke volume were increased by the same magnitude by concomitant administration of LU and Tr (Figure 4).

Cardiac Hemodynamics
In CHF rats, compared with sham animals, LVSP and LV dP/dt\textsubscript{max} were significantly reduced, whereas LVEDP was increased. LU and Tr reduced LVSP and LVEDP without affecting LV dP/dt\textsubscript{max} after 3 or 9 months of treatment. Compared with Tr alone, concomitant administration of LU and Tr additionally reduced LVSP and LVEDP without affecting dP/dt\textsubscript{max} (Figure 5).

Cardiac Morphology
Compared with sham rats, heart weight and LV collagen density were significantly increased in CHF animals. Although LU reduced heart weight but not collagen density, chronic treatment with Tr or LU + Tr reduced both heart weight and collagen density to the same extent, both after 3 and 9 months of treatment (Figure 6).

Discussion
The present study, using a rat model of chronic heart failure, shows that combined administration of a selective ET\textsubscript{A} antagonist and an ACE inhibitor reduces cardiac preload and afterload and increases stroke volume more markedly than the ETA receptor antagonism or the ACE inhibitor alone, whereas only combined administration slightly reduces heart rate. Furthermore, concomitant ETA antagonism and ACE inhibition prevented left ventricular remodeling to the same extent as each individual treatment. Finally, despite the hemodynamic and structural effects, selective ET\textsubscript{A} receptor blockade alone did not affect survival, while the additive hemodynamic effects of combined ET\textsubscript{A} receptor blockade with ACE inhibition did not modify the improvement of long-term survival compared with ACE inhibition alone.

The rat model of coronary artery ligation has been extensively used, not only to elucidate the main features of CHF but also to study the effects of pharmacological treatments, among which are ACE inhibitors and ET antagonists.9,16,22,23 In our experiments, untreated animals develop severe CHF, evidenced by an augmented LVEDP and LV cavity dilatation, as well as a progressive deterioration of LV function assessed by serial echocardiography.

As already reported with selective ET\textsubscript{A}, mixed ET\textsubscript{A}-ET\textsubscript{B} receptor blockers,8,23,24 and ACE inhibitors,22,25,26 LU 135252 and trandolapril decrease arterial blood pressure, improve cardiac hemodynamics, limit left ventricular remodeling, and increase both fractional shortening and posterior wall thickening. This, together with the decrease in cardiac preload and afterload, results in an increased stroke volume and cardiac
output and prevents the deterioration of global LV function, without affecting LV dP/dtmax.

These hemodynamic and structural effects were associated in the case of trandolapril, but not LU 135252, with an improvement of survival. Whereas the beneficial effect on survival of ACE inhibitors are well established,16,22,27,28 the effects of ET receptor blockers are contradictory. Indeed, mixed ET_A-ET_B antagonists have been shown to improve survival7,9 or to be deleterious,18 whereas ET_A antagonists have been reported to improve7,10 or have no effect on long-term survival in experimental congestive heart failure.11 Adverse left ventricular remodeling, especially attributable to acute distension of the infarcted part of the left ventricular remodeling during the healing phase after the coronary artery ligation, has been evoked to explain the absence or even detrimental effects on survival when treatment is started early after induction of myocardial infarction (~24 hours after ligation).29 However, this hypothesis can be excluded in our study, because LU treatment started after completion of the scar formation, ie, 7 days after ligation, and also prevented late remodeling of the viable part of the left ventricle. As stated above, the peptide ET_A antagonist BQ-123 and the nonpeptide ET_A antagonist TA-0201 improve survival in rats with coronary artery ligation or in cardiomyopathic hamsters, respectively.7,10 However, the ET_A-ET_B receptor ratio and distribution might be species dependent, which would explain that the effects of ET antagonists might vary according to the animal strain used. Moreover, the selectively for ET_A receptor
tors of BQ-123 and TA-0201 is based on in vitro results using cloned human ET_A and ET_B receptors, but it might be possible that both drugs at the dose used in the in vivo studies act as mixed ET_A-ET_B antagonists, the latter having shown to improve long-term survival in CHF. In our study, LU 135252 is used at a dose that selectively blocks ET_A receptors, because the dose of 30 mg/kg per day blocks the ET_A-mediated vasoconstrictor response without modifying the ET_B-mediated dilator response.8 Finally, the duration of the treatment period is extremely important when studying survival. For example, the calcium channel antagonist amlodipine improves survival during the first 6 months, whereas no improvement of survival is observed after 9 months of treatment.30 Thus, the beneficial effect of a 12-week treatment with BQ-123 or TA-0201 may not persist after a longer (9-month) treatment period.

The concomitant administration of LU and Tr attempts to reproduce the clinical context of CHF. Indeed, in most patients, any new treatment will be administered in association with an ACE inhibitor, which is today the first choice treatment of CHF in humans. In this context, concomitant administration of Tr and LU induces a more marked decrease

**Figure 3.** LV end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), and LV fractional shortening (LV Fract Short) measured in anesthetized rats with CHF, with either untreated (○) or treated by LU (□), Tr (△), or LU+Tr (★). *P<0.05 vs untreated CHF.

**Figure 4.** Cardiac index and stroke index measured in anesthetized rats with CHF, either untreated (○) or treated by LU (□), Tr (△), or LU+Tr (★). *P<0.05 vs untreated CHF.

**Figure 5.** LV systolic pressure, LV end-diastolic pressure, and LV dP/dtmax (103 mm Hg/s) measured in anesthetized sham-operated rats (3 months, n=14; 9 months, n=10; white bars) or in rats with CHF, either untreated (3 months, n=15; 9 months, n=9; black bars) or treated with LU (3 months, n=11; 9 months, n=11; up-hatched bars), Tr (3 months, n=13; 9 months, n=14; down-hatched bars), or LU+Tr (3 months, n=15; 9 months, n=12; cross-hatched bars). *P<0.05 vs untreated CHF; †P<0.05 vs Tr- or LU-treated CHF.
in blood pressure and a more pronounced reduction in heart rate than Tr or LU alone. The additive effect on systemic hemodynamics after long-term combined ET\textsubscript{A} antagonism and ACE inhibition is accompanied by an additional reduction of LVEDP and increase of stroke volume, illustrating an additional improvement of LV hemodynamics and function compared with ACE inhibition alone. This has been previously described after acute or during a 3-month treatment with selective ET\textsubscript{A} or mixed ET\textsubscript{A}-ET\textsubscript{B} antagonists in humans\textsuperscript{4,15} as well as after acute administration in animals models in rats with CHF.\textsuperscript{3}

These additive effects on cardiac hemodynamics/function of combined ET\textsubscript{A}-antagonism and ACE inhibition not only exist but also seem even more pronounced in humans. Indeed, in humans, the reduction in LVEDP after acute administration of ET antagonists seems more marked than in our experimental conditions.\textsuperscript{4,15} This could be attributable to the fact that in our study, despite the use of a low dose, Tr or LU induce already near-maximal effect in terms of cardiac hemodynamics, as suggested by the almost normalization of LVEDP, whereas their hypotensive effect is only submaximal.

Despite these additive hemodynamic effects of concomitant treatment, no additional improvement of survival was observed. Knowing that cardiac unloading and heart rate have a determinantal role in survival in this model of CHF,\textsuperscript{22} it might be possible that the synergistic effects, ie, cardiac preload and afterload reduction as well as heart rate decrease, are not marked enough to provoke an additional improvement of survival. Furthermore, the fact that the combination of LU and Tr does not additionally reduce cardiac remodeling, ie, hypertrophy, LV dilatation, and collagen accumulation, compared with ACE inhibition, might give another explanation, knowing the prognostic value of cardiac hypertrophy and dilatation in CHF.\textsuperscript{14,31} Finally, the effect of ET blockade might be less pronounced when administered in the presence of an ACE inhibitor, because of the fact that chronic ACE inhibition inhibits the activation of the ET system.\textsuperscript{32} Finally, it cannot be excluded that, in our experimental conditions, Tr already induces a maximal improvement of survival, excluding additional improvement after the combination therapy. In any case, whether a synergistic effect on long-term survival of concomitant ET\textsubscript{A} and ACE antagonism exists in humans is still unknown.

In conclusion, in this rat model of CHF, long-term combined administration of an ET\textsubscript{A} antagonist and an ACE inhibitor induces additional effects in terms of systemic and cardiac hemodynamics; however, this is not associated with an additional benefit on cardiac remodeling or increase in long-term survival. Hence, whether these additive effects of combined ET\textsubscript{A} receptor blockade/ACE inhibition are of clinical relevance in the treatment of heart failure in humans remains to be elucidated.

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References


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