Effects of a Specific Endothelin-Converting Enzyme Inhibitor on Cardiac, Renal, and Neurohumoral Functions in Congestive Heart Failure

Comparison of Effects With Those of Endothelin A Receptor Antagonism

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Background—Endothelin (ET)-1 is generated from big ET-1 by endothelin-converting enzyme (ECE). Plasma big ET-1 and ET-1 levels are strongly related to survival in patents with congestive heart failure (CHF). Because selective enzymatic processing of ET-1 formation appears to be an important therapeutic target for CHF, we investigated the acute effects of a specific ECE inhibitor on cardiorenal and endocrine functions in CHF compared with those of a selective ETA receptor antagonist.

Methods and Results—CHF was induced in beagle dogs by rapid right ventricular pacing (270 bpm, 14 days). Two incremental doses of a specific ECE inhibitor, FR901533, or a selective ETA receptor antagonist, FR139317 (1 and 3 mg/kg, n=8, respectively), were injected into dogs with CHF. FR901533 and FR139317 decreased mean arterial pressure and pulmonary capillary wedge pressure associated with reduction in systemic and pulmonary vascular resistance. These agents increased cardiac output but did not affect left ventricular fractional shortening. FR139317 exerted a greater depressor effect on mean arterial pressure than FR901533 (P<0.05). These agents decreased plasma atrial natriuretic peptide levels, but only FR901533 decreased plasma renin activity, angiotensin II, and aldosterone levels. Neither agent changed the plasma norepinephrine level despite the fall in blood pressure. These drugs increased the urinary water and sodium excretion rate associated with increases in the glomerular filtration rate and renal plasma flow, and the incremental magnitude induced by FR139317 was larger than that by FR901533 (P<0.05).

Conclusions—An ETA receptor antagonist appeared to induce greater vasodilative effects on systemic and renal vasculature in CHF than an ECE inhibitor. However, the ECE inhibitor reduced the secretion of neurohumoral factors that are activated in proportion to the severity of CHF. Our acute complementary data may support the importance of the role of ECE in CHF and provide a rationale foundation for investigating the usefulness of long-term treatment with ECE inhibitors in CHF. (Circulation. 1999;99:570-577.)

Key Words: endothelin ■ heart failure ■ enzymes ■ receptors ■ hormones

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Endothelin (ET)-1, the most powerful endothelium-derived vasoconstrictor, is generated as prepro ET-1 that is enzymatically cleaved to form an intermediate, big ET-1, and big ET-1 is further processed to form the biologically active mature ET-1 by a specific phosphoramidon-sensitive metalloprotease called endothelin-converting enzyme (ECE).1,2 Significant elevations of plasma big ET-1 and ET-1 levels are observed in congestive heart failure (CHF) compared with those in normal control subjects, and these variables are strongly related to survival in CHF patients.3,4 Indeed, long-term treatment with some selective ETA and mixed ETA/B receptor antagonists greatly improved the survival of rats with CHF.5,6 Considering the evidence that both ETA and ETB receptors mediate vasoconstriction in some vessels,7 we can anticipate an alternative option of anti-ET therapeutic strategy, that is, blocking the generation of ET-1 by an ECE inhibitor. Indeed, brachial administration of phosphoramidon, an ECE inhibitor, caused forearm vasodilation and increased forearm blood flow in patients with CHF.8 However, the compound is not a selective inhibitor; rather, it is more of a neutral endopeptidase than an ECE inhibitor, and no specific ECE inhibitors have been developed so far. Systemic administration of ECE inhibitors blocks all biologically active ET-1 actions including both ETA and ETB receptor-mediated ET-1 actions. However, we previously reported that the selective ETB receptor antagonist...
RES-701-1 increased systemic and pulmonary vascular resistance and decreased cardiac output (CO) in CHF. Therefore it should be evaluated whether there are any adverse effects from inhibiting the ET system at the level of ECE and whether specific ECE inhibition is really a useful treatment for CHF. A specific ECE inhibitor, FR901533, which has greater potency and selectivity for ECE than phosphoramidon, has been synthesized. Our principal objective was to evaluate whether the modulation of the production of ETs with the specific ECE inhibitor has beneficial effects in treating CHF, as ET receptor antagonists have shown. We examined the acute effects of FR901533 on hemodynamic, neurohumoral, and body fluid regulation by comparing those effects of a selective ETA receptor antagonist, FR139317, in dogs with CHF induced by rapid ventricular pacing.

**Methods**

**Animal Preparation**

Conditioned beagles of either sex, weighing 10 to 13 kg, were used for all experiments, as previously described. This study was approved by the Animal Research Committee of Shiga University of Medical Science. After anesthesia was induced with pentobarbital sodium (25 mg/kg), the dogs were ventilated with a respirator (Aika R-60). Through a left thoracotomy, the heart was exposed and 2 pacemaker leads (Matsuda M-23) were sutured onto the right ventricular apex. After the incision was closed, the leads were tunneled to the back and connected to an external pacemaker (Seamed 540). The left femoral vein was then exposed and a thermocatheter (Goodtec T-047-03) was advanced into the pulmonary artery to measure right atrial pressure (RAP), mean pulmonary arterial pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and CO. A Tygon catheter (Norton R3603) was placed in the descending thoracic aorta through the left femoral artery to measure mean arterial pressure (MAP). The right jugular vein was also catheterized, and the Tygon catheter was advanced into the vena cava for drug administration. All of those chronic catheters were implanted percutaneously and flushed with heparin-saline 3 times per week. The pacemaker and the ends of the catheters and pacing wires were securely fastened in a small bag worn on the back of the animal. After recovering from instrumental surgery for at least 14 days, study 1 was performed. The day after the completion of study 1, normal baseline measurements were taken and the pacemaker was programmed to an asynchronous mode at a rate of 270 bpm and pacing continued for 14 days for study 2.

CO was assessed in triplicate by the thermodilution technique with a Gould CO computer (Statham SP1435). Systemic vascular resistance was calculated as ([MAP−RAP]×80)/CO and pulmonary vascular resistance as ([MPAP−PCWP]/PCWP). A 2-dimensional and M-mode echocardiogram (Toshiba SAL-77A, 2.5-MHz transducer) was performed from a right parasternal approach to image the left ventricle. The left ventricular (LV) fractional shortening was calculated as the ratio of \([(LV\text{ end-diastolic diameter}−LV\text{ end-systolic diameter})/LV\text{ end-diastolic diameter}]×100\). Blood was drawn from the pulmonary artery through the thermodilution catheter and transferred to specially prepared tubes stored on ice for analysis of plasma big-ET-1, ET-1, atrial natriuretic peptide (ANP), angiotensin II, aldosterone, and norepinephrine concentrations and plasma renin activity (PRA). The blood specimens were centrifuged at 4°C and the plasma was frozen at −30°C until assay. All subsequent studies were performed with animals in the conscious state.

**Experimental Protocol**

**Study 1: Inhibitory Pressor Effects of FR901533 in Response to Big ET-1 and ET-1**

The selective nature of FR901533 as an ECE inhibitor has been demonstrated to be at least 3 times more potent than phosphoramidon in ECE inhibition of bovine endothelial cells and to inhibit both ECE-1 and ECE-2 activities with similar potencies. FR901533 interacted with metalloenzyme at nanomolar concentrations and did not inhibit neutral endopeptidase and collagenase activities, whereas phosphoramidon is ~50 times more active against neutral endopeptidase than ECE.

To confirm the in vivo effects of FR901533 as an ECE inhibitor, we observed the inhibitory effects of it on MAP responses to exogenously administered human big ET-1 or ET-1 (Peptide Institute). The criterion for the initial dose selection of FR901533 was to block the pressor response to big ET-1 that produced a rise in MAP of ≥15 mm Hg from the pretreatment blood pressure. Eight dogs were randomly selected and studied after complete recovery from the effects of surgery. After the hemodynamics had stabilized, big ET-1 (0.2 nmol/kg, n=4) or ET-1 (0.75 nmol/kg, n=4) alone was injected as a bolus. After 3 days of the first study, FR901533 (Fujisawa Pharmaceutical Co, Ltd, 1 mg/kg) was administered to each dog 5 minutes before big ET-1 or ET-1 was injected. Changes in MAP in response to big ET-1 or ET-1 were recorded for 60 minutes in each group without rapid pacing.

**Study 2: Cardiorenal and Hormonal Effects of ECE Inhibitor and ETA Antagonist in Dogs With CHF**

After inducing CHF with 14 days of rapid pacing, we compared the acute effects of the ECE inhibitor with those of selective ETA receptor antagonist on the changes in hemodynamics, hormones, and
renal functions in CHF. We divided the dogs with CHF into 3 groups; the ECE inhibitor group (n=8) was given FR901533, the ETA block group (n=8) was administered a selective ETA receptor antagonist, FR139317 (Fujisawa Pharmaceutical Co, Ltd), and the vehicle group (n=5) received only saline as a control. All subsequent measurements were recorded with ongoing rapid ventricular pacing. As previously reported, the urinary bladder was catheterized with a CLINY balloon tube (Create Medic Co) under brief thiopental sodium anesthesia (4 mg/kg); 45 minutes later, a priming dose of 50 mg/kg para-aminohippurate dissolved in a 10-ml saline solution was infused over a period of 10 minutes. During each clearance period, blood sampling were performed at the end of each clearance period, each of which lasted for 20 minutes. After 2 sequential baseline periods, in the ECE inhibitor group, the first bolus dose of FR901533 at 1 mg/kg dissolved in a 20-ml saline solution was administered intravenously. After 30 minutes, the second dose of 3 mg/kg was injected, and two 30-minute clearances were allowed for each dose. In the ETA block group, FR139317 was administered at 1 and 3 mg/kg, given at intervals of 30 minutes in the same way. FR139317 at a dose of >1 mg/kg has previously been shown to inhibit endogenous ET-1 activity through ETA receptors. In the vehicle group, saline solution was administered at intervals of 30 minutes to exclude any temporal effects. During each clearance period, the pressure parameters were recorded every 5 minutes. CO and blood sampling were performed at the end of each clearance period. An echocardiogram was performed to estimate changes in LV fractional shortening at the baseline and the end of all the experiments during a brief cessation of rapid pacing.

**Analysis of Blood and Urine Samples**

The plasma big ET-1 concentration was measured by ELISA with a commercial kit (Biomedica Gesellschaft). Plasma (500 μL) was precipitated and dried in a Speed Vac concentrator (Savant SC110A). Peptides were reconstituted with 500 μL of assay buffer and were subjected to ELISA. One hundred microliters of samples and 50 μL of monoclonal detection antibody were simultaneously added to the kit wells precoated with a polyclonal rabbit antibody to human big ET-1. Big ET-1 was quantitated by an enzyme-catalyzed color change detectable on the ELISA reader (Titertek Multiskan MCC/340 MK II) at 450 nm filter. The intra-assay and interassay coefficients of variation were 3.9% (n=11) and 6.1% (n=12). The plasma ET-1 concentration was determined by radioimmunoassay as previously described. Blood for PRA, angiotensin II, aldosterone, and ANP assay was measured by radioimmunoassay as previously described. Plasma norepinephrine concentration was performed by high-performance liquid chromatography, as previously reported. Serum and urine creatinine, para-aminomimidopurine, and sodium concentrations were measured as previously reported. Creatinine clearance and para-aminomimidopurine clearance were calculated with standard formulas and were equated with glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively.

**Statistical Analysis**

All data are presented as mean±SEM. ANOVA for repeated measurements was used to determine the significance of changes during multiple time-dependent observations. Comparisons with baseline values were analyzed by Dunnett’s test after ANOVA for repeated measurements. Student’s t test was used to analyze the significance of single comparisons. A value of P<0.05 was considered significant.

**Results**

**Blocking Effects of FR901533 on Big-ET-1 and ET-1 Injection**

The effects of FR901533 on big ET-1–induced or ET-1–induced changes in MAP are shown in Figure 1. ET-1 elicited an initial transient depressor response followed by sustained hypertension, whereas big ET-1 induced a prolonged pressor response similar to that induced by ET-1, but there was no initial transient depressor effect. Pretreatment with FR901533 significantly blocked the big ET-1–induced pressor response, but FR901533 did not affect the ET-1–induced initial hypotensive and subsequent hypertensive effects.

**Characteristics of CHF**

Hemodynamic and endocrine characteristics in normal dogs, just before initiation of rapid ventricular pacing and again after induction of CHF, are summarized in Table 1. After 2 weeks of rapid pacing, PCWP and RAP were significantly increased, but MAP and CO were decreased relative to the respective normal values in all the groups. The LV fractional shortening was also decreased compared with that in normal
Hemodynamic Effects of ECE Inhibitor and ETA Block in CHF

Hemodynamic responses to FR901533 and FR139317 are shown in Figure 4. Both compounds caused significant decreases in plasma ANP levels with modest reductions in PCWP. Only FR901533 significantly decreased PRA (P<0.05), angiotensin II (P<0.05), and aldosterone levels (P<0.01). Despite the decrease in blood pressure, neither drug changed the plasma norepinephrine levels from 811±102 to 710±94 and from 780±141 to 742±103 pg/mL, respectively.

Renal Effects of ECE Inhibitor and ETA Block in CHF

The effects of FR901533 and FR139317 on renal functions are shown in Table 2. Both compounds significantly increased the urine flow rate and absolute urinary sodium excretion compared with the average basal values. These agents also significantly increased GFR and RPF; however, FR139317 caused greater increases in those values than FR901533 (P<0.05).

Discussion

Considering that anti-CHF therapy is not totally successful in reducing mortality rates, the ET system is an attractive target for therapeutic intervention in CHF because long-term treatment with ET receptor antagonists has improved survival in rats with CHF. However, it was reported that the density and affinity of ET receptors were altered in CHF, and long-term treatment with ET receptor antagonists may not always exert consistent efficacy in CHF. Another area of research on alternative anti-ET agents involves ECE inhibition. The identification of the potential usefulness of ECE inhibition in CHF is an important objective of our study compared with the acute effects of an ETA receptor antagonist on hemodynamics, neurohumoral functions, and body fluid balance.

Although there is no direct index showing the inhibition of conversion of big ET-1 to ET-1 in vivo, ECE inhibition may theoretically increase plasma big ET-1 and decrease plasma ET-1 levels. When we administered FR901533, which blunted the big ET-1-induced pressor effect, the apparent rise in plasma big ET-1 levels can serve as a marker of considerable inhibition of conversion to ET-1 in intact animals. Failure of plasma ET-1 level to decrease with ECE inhibitor may be due do the oversaturated clearing capacity or the downregulation of ETB receptors, which mediated endocytosis of ET-1. A longer duration of ECE inhibition may be required to lower plasma level of ET-1.

Changes in Hemodynamics in CHF

An ECE inhibitor, phosphoramidon reduced pulmonary hypertension after cardiopulmonary bypass in pigs and it caused substantial forearm vasodilatation in patients with
However, the vasodilative action of phosphoramidon could be due, in part, to the effect of ANP as a result of inhibition of neutral endopeptidase activity. Since we demonstrated that an important vasodilative action involving ETB receptors exists in CHF and that a selective ETB receptor blockade could be harmful to hemodynamics, it is necessary to examine the effects of specific ECE inhibitors on hemodynamics. FR901533 slightly but significantly reduced MAP and PCWP in association with a decrease in systemic and pulmonary vascular resistance. These results suggest that the ET system overall acts as a vasoconstrictor in CHF. However, an ETA receptor antagonist, FR139317, caused a greater decrease in MAP than did FR901533. This may be due to the fact that the ECE inhibitor blocked the vasodilative effect mediated by ETB receptors and/or the ETA receptor antagonist blocked the vasoconstrictive effect of ET-1 and might simultaneously augment the vasodilative effect through the ETB receptor. Because ET-1 has a positive inotropic effect on cardiac muscle, both compounds theoretically elicit a negative inotropic effect. However, these agents did not change LV fractional shortening in the present study. Thomas et al. and Spinale et al. reported that ET-1 caused a dose-dependent decrease in myocyte contractile function and that long-term administration of ETA receptor antagonist improved isolated myocyte contractility and normalized inotropic responsiveness in rapid pacing–induced CHF. Both compounds may alter loading conditions and produced greater hemodynamic beneficial effects; however, long-term investigation is needed to evaluate whether the anti-ET therapy has beneficial effects on myocyte contractile function in CHF.

Changes in Neurohumoral Factors in CHF

To determine whether ECE inhibition has a therapeutic potential in treating CHF, it might be helpful to assess the effects on neurohumoral integration of the ET system. If FR901533 is merely a vasodilator, it will result in reflex activation of the vasoconstrictive endocrine system. However, the agent did not change the plasma norepinephrine levels despite a fall in blood pressure. Furthermore, the ECE inhibitor but not the ETA receptor blocker significantly decreased PRA, angiotensin II, and aldosterone levels. Although ET-1 directly inhibited renin release by increasing calcium influx in juxtaglomerular cells, the decrease in PRA appeared to be mediated by both the macula densa mechanism and increased RPF in the present study. In contrast, ET-1 enhanced the conversion of angiotensin I to angiotensin II in cultured endothelial cells and directly stimulated aldosterone secretion in the adrenal zona glomerulosa. Despite blocking the beneficial ETB receptor–mediated vasodilative action, the fact that FR901533 reduced the vascular resistance may partly involve the subsequent decrease in angiotensin II–mediated vasoconstriction. ET-1 appeared to be involved in the complicated modulation of the renin-angiotensin-aldosterone (RAA) system, and further studies are required to address whether the ET system directly regulated secretions of
the RAA system through ECE or ETB receptors. Because those neurohumoral factors are activated in proportion to the severity of CHF and are prognostic indicators in patients with CHF, the decline in the hormone levels with FR901533 may indicate that an ECE inhibitor can attenuate aggravation of CHF.

Changes in Renal Functions in CHF
Exogenous ET-1 increases renal vascular resistance and falls in RPF and GFR, thereby reducing urine production. We demonstrated that an ETA receptor antagonist improved renal hemodynamics as well as water and sodium, whereas an ETB receptor antagonist decreased RPF in this canine model. In rats with ischemic renal failure, phosphoramidon restored the GFR, ameliorating the reduction of urine flow and RPF. In the present study, FR901533 significantly increased the urinary flow rate, sodium excretion rate, GFR, and RPF, similar to the effects induced by FR139317. An ECE inhibitor may be useful in treating body fluid retention associated with CHF; however, the renal vasodilative effect of an ETA receptor antagonist appeared to be greater than that of an ECE inhibitor, similar to its effect on the systemic vasculature. Sodium and water excretion are also regulated by ANP and the RAA system. Although both compounds decreased the plasma ANP level, the increases in RPF and GFR may have exceeded the reduction in ANP-induced diuresis and natriuresis. Because angiotensin II reduces RPF by vasoconstriction and angiotensin II and aldosterone regulate extracellular fluid volume by their effects on sodium retention, the improved renal functions with ECE inhibition might partly be due to suppression of the RAA system.

Limitations of the Study
There are several limitations of the present study. First, because the development of tachycardia-induced CHF is reversible and the termination of rapid pacing resulted in

![Figure 4. Hormonal effects of FR901533 (closed bars, n=8), FR139317 (open bars, n=8), and vehicle saline (hatched bars, n=5) on plasma ANP, renin activity, angiotensin II, and aldosterone concentrations in dogs with heart failure. Two bolus doses of FR901533, FR139317 (1 and 3 mg/kg, respectively), or saline vehicle were given at intervals of 30 minutes. *P<0.05, †P<0.01 compared with baseline.]

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<th>TABLE 2. Comparison of Renal Effects of ECE Inhibitor and ETA Receptor Antagonist in Dogs With Heart Failure</th>
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<td><strong>ECEI Group (n=8)</strong></td>
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ECEI indicates ECE inhibitor; U-V, urinary flow rate; and U-Na, urinary sodium excretion.

After two 20-minute basal clearance periods, the ECEI group was given FR901533 (1 and 3 mg/kg), the ETA Block group was given FR139317 (1 and 3 mg/kg), and the vehicle group was given only saline at 30-minute intervals. Two 30-minute clearances were performed after administration of each dose. Baseline values are average obtained during preceding basal period. Values represent mean±SEM.

*P<0.05.
†P<0.01 vs baseline.
‡P<0.05 vs ECEI group.
improvement of cardiac and hormonal functions, it would have become difficult to distinguish changes caused by the ECE inhibitor and ETA receptor antagonist from the effects of deactivating the pacing. Thus we performed these experiments with ongoing rapid pacing. However, rapid ventricular pacing is associated with markedly asynchronous ventricular wall motion, rendering accurate M-mode echocardiographic measurements difficult. Therefore, we measured the LV fractional shortening during sinus rhythm only. Second, the geometric shape of the LV cavity in our tachycardia-induced CHF model is far more complicated than a sphere and, even after the termination of pacing, there is a problem involving the validity of LV volume calculations. Although LV fractional shortening is one way of describing the quality of LV contractions, it does not equate to the stroke volume measured by the thermodilution technique. Third, a higher dose of FR901533 was expected to exert more favorable effects; however, it did not induce any further effects on MAP or PCWP. We were concerned about excessive hypotension, which would cause reflex activation of the vasoconstrictive hormones and decreased urine output if we administered a much higher dose of FR901533. FR901533 is an expensive and scarce compound; therefore, we could not evaluate the long-term or the higher dose–effects in large animals.

In conclusion, because CHF is a chronic disease, the therapeutic modality of ECE inhibition should be evaluated by long-term treatment of a specific ECE inhibitor, not the short-term effects. However, to our knowledge, it has not been demonstrated that specific ECE inhibition had acute beneficial effects on cardioenrical and neurohumoral functions in CHF. An ETA receptor antagonist appeared to induce greater vasodilative effects on systemic and renal vasculature in CHF than ECE inhibition. However, the ECE inhibitor reduced the secretion of neurohumoral factors, which are activated in proportion to the severity of CHF. Therefore, even though the vasodilative effect of an ECE inhibitor may be less than that of an ETA receptor antagonist, ECE inhibition may have demonstrated favorable potential in dogs with CHF. Our complementary data may support the importance of the role of ECE in generating ET-1 in CHF and provide a rational foundation for investigating the usefulness of long-term treatment with ECE inhibitors in CHF.

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References


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