Original Article

Acute Effects of E-3174, a Human Active Metabolite of Losartan, on the Cardiovascular System in Tachycardia-Induced Canine Heart Failure

Jun SUZUKI, Hisashi OHTA, Kayoko HANADA, Nobuko KAWAI, Takanori IKEDA, Mizuki NAKAO, Fumihiko IKEMOTO*, and Masaru NISHIKIBE

The aim of this study was to evaluate the acute effects of E-3174, a human active metabolite of the AT1 receptor antagonist, losartan, on hemodynamic functions in dogs with severe heart failure (HF). In dogs, insignificant plasma levels of E-3174 are present following administration of losartan, and therefore, the effects of these two drugs can be studied independently in the dog. HF was established by rapid pacing of the right ventricle (250-270 beats/min) for 4 weeks. We examined changes in cardiovascular functions after acute intravenous administration of losartan (1 mg/kg) and E-3174 (0.3 and 1 mg/kg), as well as an ACE inhibitor, enalapril (0.3 and 1 mg/kg), under condition of HF. The HF before treatment was characterized by increases in pre- and after-load of the left ventricle (LV), consequent low cardiac output, and LV dilatation. E-3174 at 0.3 and 1 mg/kg reduced pulmonary artery pressure (−13±6% and −22±3% from baseline, respectively, p<0.05), pulmonary capillary wedge pressure (−18±4% and −36±10%, p<0.05) and mean arterial pressure (−24±2% and −36±7%, p<0.05), increased stroke volume (SV: +12±7% p>0.05; +36±19%, p<0.05), and reduced peripheral resistance (−23±5% and −41±9%, p<0.05), but had no effect on the first derivative of left ventricular pressure (dP/dt/P) or the time constant for relaxation. Effects of losartan at 1 mg/kg were similar to those of 0.3 mg/kg of E-3174. Enalapril at 1 mg/kg caused changes comparable to those seen after E-3174 administration (1 mg/kg), except that the increase in SV (+16±8%, p<0.05) with enalapril was not as great as that with E-3174. Both losartan at 1 mg/kg and E-3174 at 0.3 and 1 mg/kg increased fractional shortening to a similar extent (FS: +52±12%, +47±8% and +56±8%), while enalapril at 0.3 and 1 mg/kg had no significant effects on FS. Reflex elevation of plasma renin activity induced by 1 mg/kg of E-3174 was similar to that caused by 1 mg/kg of enalapril, suggesting that the two drugs achieved similar inhibition of the endogenous renin angiotensin system. Our study demonstrated that acute blockade of the AT1 receptor with E-3174 reduced elevated pre- and after-load and consequently increased stroke volume in a canine HF model. With the exception of changes in stroke volume, these effects of E-3174 were comparable to those produced by enalapril, and were 3 times stronger than those by losartan. (Hypertens Res 2001; 24: 65–74)

Key Words: angiotensin II, E-3174, losartan, enalapril, canine heart failure

Introduction

The renin-angiotensin system (RAS) is one of the neuro-hormonal systems that is activated in chronic heart failure (CHF; 1-3). Increased activity of the RAS is maintained throughout the development of CHF (4, 5). It has been shown that blockade of activated RAS by angiotensin

From the Pharmacology, Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., Tsukuba, Japan, and *Development Research Laboratories, Banyu Pharmaceutical Co., Ltd., Ibaraki, Japan.
Address for Reprints: Jun Suzuki, Ph.D., Pharmacology, Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., 3 Okubo, Tsukuba, Ibaraki 300-2611, Japan.
Received July 19, 2000; Accepted in revised form October 12, 2000.
converting enzyme inhibitors (ACEIs) is an effective treatment in clinical CHF trials (6, 7). The effectiveness of ACEIs has been also shown in many experimental models of CHF. Acute treatment with a reduced total peripheral resistance (TPR) and an increased cardiac output (CO) in canine CHF induced by right ventricular rapid pacing (5, 8) as well as rat CHF induced by myocardial infarction (9). Chronic treatment with an ACEI was also effective in reducing mortality in a rat model (10) and delayed the development of CHF in a canine model (8).

Recently, blockade of the angiotensin receptor, particularly selective blockade of the angiotensin II type 1 (AT1) receptor by losartan, has been shown to result in a mortality reduction similar to that by captopril in clinical trials with CHF patients (11). Therefore, continuous stimulation of the AT1 receptor by elevated angiotensin II (AII) contributes to deterioration of cardiovascular function in CHF patients. In contrast to its clinical effectiveness, losartan in experimental CHF models does not always exhibit effects comparable to those of ACEIs (5, 8, 12).

CHF elicited by sustained tachycardia in experimental animals shares a number of features — such as progressive ventricular dilation — in common with dilated cardiomyopathy in humans (13-15). Both patients with dilated cardiomyopathy and dogs with pacing-induced CHF show reduced cardiac output. However, CHF elicited by tachycardia in experimental animals generally demonstrates decreased systemic arterial pressure with no increase in total peripheral vascular resistance, while in CHF patients, total peripheral resistance is increased and systemic blood pressure may not be reduced until the late stage of CHF (6, 7, 11). Therefore, activation of the AT1 receptor by AII may play different roles in clinical and experimental CHF. In the case of losartan, another possible difference may be observed between CHF in men and dogs due to species-dependent differences in losartan metabolism. It is known that losartan is rapidly metabolized to a potent active metabolite, E-3174, in humans but not in dogs (16-18). E-3174 is 15 times more potent than losartan for blocking AII-evoked pressor responses in vivo. In vitro, the potency of E-3174 for blocking AT1 receptor was more than 30 times stronger than that of losartan (19, 20). Therefore, dog models of CHF provide an opportunity to characterize the direct effects of E-3174 on hemodynamics in CHF, and to compare the effects of E-3174 with those of losartan and enalapril.

The goal of this study was to determine whether an acute AT1 blockade could cause an effective reduction of the pre- and after-load of the ventricle and subsequent increases in cardiac performance in an established model of heart failure. The results of this study will provide further insight into the roles of RAS and AT1 receptors in CHF and the clinical relevance of losartan in the treatment of CHF patients.

Materials and Methods

Chronic Heart Failure (CHF) Model with Rapid Right Ventricular Pacing (RRVP)

Forty-one adult male beagle dogs weighing 10-14 kg were used for the experiments. All experimental procedures were approved by in-house animal use committees and followed the guidelines of the Japanese Pharmacological Society. Before surgery, blood was taken from the cephalic vein for determination of plasma renin activity (PRA), aldosterone (ALDO), atrial natriuretic peptide (ANP), and norepinephrine (NE) levels. All surgical procedures were performed under aseptic conditions. Under general anesthesia induced and maintained by isoflurane (5 and 2 vol%, respectively) with nitrous oxide (3 l/min) and oxygen (2 l/min), artificial ventilation was performed through a tracheal tube (rate, 15 times/min; volume, 300 ml) to maintain appropriate blood gases. After an at least 30-min stabilizing period, an echocardiogram was recorded for determination of basal conditions. Thereafter, a bipolar pacemaker electrode (3272VB, Aisne Productions Cardiologiques Int., Thierry, France) was advanced through the branch of the right external jugular vein and positioned in the right ventricle. The other end of the electrode was tunneled to a subcutaneous pocket in the left lateral neck and connected to a pulse generator (SJP-501, Star Medical, Tokyo, Japan), which was buried in the subcutaneous pocket. The animals received an antibiotic for up to three days after the implantation of electrodes, and at 1 to 2 weeks after surgery, pacing was initiated at a rate of 250 to 270 beats/min. The dogs were examined frequently to confirm the maintenance of pacing and to monitor for incipient symptoms of CHF. The pacing was stopped when signs of CHF (ascites, decreased motor activity and appetite, and abnormal breathing) were apparent (approximately 4 weeks of pacing; range, 3 to 5 weeks). At this time, blood was withdrawn for measurement of the hemodynamic factors mentioned above. Eight animals with RRVP were lost in this study due to sudden death during pacing.

Study Protocol

Five of 33 dogs that had LVEDP values less than 15 mmHg were excluded from the study. Baseline hemodynamic variables in the remaining 28 experimental animals and 8 normal animals are shown in Table 1.

Drug treatment and hemodynamic examinations were conducted under spontaneous sinus rhythm after cessation of pacing and an overnight rest. This protocol maintained the stable condition of the hemodynamic variables in the vehicle-treated animals throughout the observation
Table 1. Baseline Hemodynamic Variables

<table>
<thead>
<tr>
<th></th>
<th>CHF dogs (n=28)</th>
<th>Normal dogs (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>121±4</td>
<td>129±7</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>77±3*</td>
<td>100±2</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>25.4±1.1*</td>
<td>4.4±1.1</td>
</tr>
<tr>
<td>Ppa (mmHg)</td>
<td>40.6±1.8*</td>
<td>28.0±0.5</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>26.7±1.2*</td>
<td>16.3±0.5</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>9.5±0.6*</td>
<td>12.4±0.5</td>
</tr>
<tr>
<td>LV systolic wall stress (g/cm²)</td>
<td>209±11*</td>
<td>130±7</td>
</tr>
</tbody>
</table>

*n*: number of animals. HR, heart rate; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; Ppa, pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; SV, stroke volume. *p<0.05 vs. normal dogs (Student’s t-test).

period.

Baseline hemodynamic and echocardiographic parameters were measured before drug treatment. Then, a bolus injection of losartan (1 mg/ml/kg, *n*=5), E-3174 (0.3 and 1 mg/ml/kg, *n*=3 and 5), enalapril (0.3 and 1 mg/ml/kg, *n*=3 and 5), or vehicle (saline or citric/phosphate buffer, *n*=7) was administered via the femoral vein by a 10-min infusion. Hemodynamic measurements were repeated at 0 (immediately after drug injection), 10, 20, 30, 60, 90 and 120 min after drug injection. Echocardiograms were recorded before and 120 min after the injection. Blood was taken before and 120 min after the injection for determination of the PRA. Blood gas was periodically monitored in order to adjust the artificial ventilation.

Hemodynamic Measurements

On the day of the study, each animal was anesthetized and artificially ventilated as described above. A polyethylene catheter was inserted in the left femoral artery and connected to a pressure transducer (TP-400T, Nihon Kohden Inst., Tokyo, Japan) to measure mean arterial pressure (MAP). Blood samples were obtained via the arterial catheter. A polyethylene catheter was also inserted in the left femoral vein to inject each drug. A 5- or 7-F Swan-Ganz pulmonary artery catheter was positioned in the pulmonary artery through the left external jugular vein, and connected to pressure transducers for measurement of pulmonary arterial pressure (Ppa), pulmonary capillary wedge pressure (PCWP), and right arterial pressure (Pra). Cardiac output (CO) was measured in triplicate by the thermodilution method using a CO computer (MTC-6210, Nihon Kohden Inst.). A 5-F high-fidelity manometer-tipped catheter (Millar Inst, Houston, USA) was positioned in the left ventricle through the left carotid artery for measurement of LVEDP, and LV pressure-derived indices of contractility and relaxation. Transient doppler flow probes were attached to the right femoral artery and the left renal artery, and connected to blood flow meters (T206, Transonic System, Inc., Ithaca, USA) to measure femoral (FFB) and renal blood flow (RBF).

A cardiotachometer triggered by limb lead II ECG provided a continuous recording of heart rate (HR). All pressures and HR were monitored and recorded by a polygraph system (RM-6000, Nihon Kohden Inst.).

Stroke volume (SV) was calculated as the quotient of CO and HR. Total peripheral resistance (TPR) was calculated as the quotient of MAP and CO. Pulmonary vascular resistance (PVR) was calculated as the quotient of mean pulmonary pressure and CO.

LV systolic wall stress (circumferential, global average wall stress in g/cm²) was estimated according to the following formula: \( WS = (P/D^4h/[1+h/D]) \times 1.36 \), where \( P \) is LV systolic pressure, \( D \) is LV end-diastolic inner diameter, and \( h \) is LV end-diastolic posterior wall thickness (2). LV contractile function was estimated by the pressure-derived isovolumetric phase indices of the first derivative of LV pressure corrected for pressure (dP/dt/P). LV relaxation was characterized by the relaxation time constant, \( \tau \), obtained according to the method of Weiss (21).

Echocardiographic Measurements

Echocardiograms were recorded in some animals. Two-dimensionally directed (2-D) M-mode echocardiograms were obtained via the right parasternal approach with an ultrasound system (probe: 3.75 MHz, SSA-340A, Toshiba Medical Systems, Tokyo, Japan). LV cross-sectional views from the short-axis were taken at the chordal level (15, 22, 23). In M-mode echocardiograms, the LV end-diastolic inner diameter (EDD) and posterior wall thickness, and LV end-systolic inner diameter (ESD) were measured. Fractional shortening (FS) was calculated as \( 100 \times (EDD - ESD)/EDD \).

Measurement of Humoral Factors

Plasma samples were obtained from the cephalic vein before and at the end of pacing, while the animals were conscious. The samples were stored at −40°C until the assay was performed. PRA was determined by radioimmunoassay (RIA) using kits (Dinabot Radiosotope Inst., Tokyo, Japan). ALDO was estimated by RIA using a kit (Diagnostic Products Co., Tokyo, Japan). ANP was determined by enzyme immunoassay using a kit (Wako Pure Chemical Co., Ltd., Osaka, Japan). Plasma norepinephrine (NE) was measured by high-performance liquid chromatography with electrochemical detection.
Table 2. Changes in Echocardiographic Measurements and Humoral Factors after Chronic Rapid Ventricular Pacing in Dogs

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Before pacing</th>
<th>End of pacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDD (mm)</td>
<td>23</td>
<td>31.2±0.8</td>
<td>40.9±0.8*</td>
</tr>
<tr>
<td>Wall thickness (mm)</td>
<td>23</td>
<td>7.5±0.3</td>
<td>5.9±0.3*</td>
</tr>
<tr>
<td>FS (%)</td>
<td>23</td>
<td>23.5±1.2</td>
<td>10.4±0.6*</td>
</tr>
<tr>
<td>PRA (ng AI/ml/h)</td>
<td>19</td>
<td>0.69±0.14</td>
<td>3.90±1.34*</td>
</tr>
<tr>
<td>ALDO (pg/ml)</td>
<td>19</td>
<td>54±11</td>
<td>341±147*</td>
</tr>
<tr>
<td>ANP (pg/ml)</td>
<td>19</td>
<td>136±19</td>
<td>442±68*</td>
</tr>
<tr>
<td>NE (pg/ml)</td>
<td>17</td>
<td>451±29</td>
<td>1,836±320*</td>
</tr>
</tbody>
</table>

n, number of animals; EDD, left ventricular end-diastolic inner diameter; wall thickness, left ventricular end-diastolic posterior wall thickness; FS, fractional shortening; PRA, plasma renin activity; ALDO, plasma aldosterone concentration; ANP, plasma atrial natriuretic peptide concentration; NE, plasma norepinephrine concentration. *p<0.05 vs. before pacing (paired t-test).

Table 3. Effects of Losartan, E-3174 and Enalapril on Plasma Renin Activity (ng AI/ml/h) in Anesthetized Dogs with CHF

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Pre-injection</th>
<th>Post-injection (120 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>12.2±2.8</td>
<td>11.7±2.1</td>
</tr>
<tr>
<td>Losartan (1 mg/kg)</td>
<td>5</td>
<td>8.2±3.5</td>
<td>14.2±3.0*</td>
</tr>
<tr>
<td>E-3174 (0.3 mg/kg)</td>
<td>3</td>
<td>9.1±2.9</td>
<td>16.7±2.5*</td>
</tr>
<tr>
<td>E-3174 (1 mg/kg)</td>
<td>4</td>
<td>10.6±4.3</td>
<td>20.5±4.1*</td>
</tr>
<tr>
<td>Enalapril (0.3 mg/kg)</td>
<td>3</td>
<td>16.9±4.6</td>
<td>24.6±5.1*</td>
</tr>
<tr>
<td>Enalapril (1 mg/kg)</td>
<td>5</td>
<td>13.5±2.0</td>
<td>27.7±4.0*</td>
</tr>
</tbody>
</table>

n: number of animals, *p<0.05 vs. pre-injection (paired t-test).

Chemicals

The non-peptide AT1 antagonists, losartan and E-3174 (23, 24), were obtained from Merck Research Lab. (West Point, PA, USA). The ACE inhibitor, enalapril (25, 26), was obtained in-house. The antibiotic, cephalmine, was purchased from Fujisawa Pharmaceutical Co. (Tokyo, Japan). Losartan and enalapril were dissolved in isotonic saline. E-3174 was dissolved in citric acid and phosphate buffer.

Statistics

All data were reported as the mean±SEM. The statistical analyses were performed using an SAS package. The data before and after development of CHF were compared using a paired t-test. The comparisons among data for the drug and vehicle groups were made with Dunnett's test. The respective lower doses of E-3174 and enalapril were employed for evaluation of dose response. Statistical significance was considered to be achieved at p<0.05.

Results

Basal Variables in CHF

At that point in time when each dog showed symptoms of CHF, the baseline characteristics of hemodynamic and echocardiographic parameters were determined. Compared with our historical controls, the CHF dogs demonstrated typical hemodynamics after right ventricle rapid pacing: i.e., reductions in MAP and SV and increases in LVEDP, PCWP, Ppa and LV systolic wall stress (Table 1). The echocardiogram showed enlargement of LVEDD (+31% from 31.2±0.8 mm before pacing, p<0.05) and decreases in posterior wall thickness (−21% from 7.5±0.3 mm, p<0.05) and FS (−56% from 23.5±1.2%, p<0.05) (Table 2).

Accompanying the morphological and functional changes in the CHF dogs, remarkable increases in the plasma levels of PRA (5.7 times from pre-pacing value, p<0.05), ALDO (6.3 times from pre-pacing value, p<0.05), ANP (3.3 times from pre-pacing value, p<0.05) and NE (4.1 times from pre-pacing value, p<0.05) were observed at the end of the pacing period (Table 2).

PRA Changes Related to Drug Treatment

Results of the PRA measurements before and 120 min after treatment for the six groups are presented in Table 3. There was no significant difference in baseline values among the six groups. Significant increases in PRA were induced by both intravenous E-3174 at 0.3 and 1 mg/kg (+84% and +94%, p<0.05 from the respective pre-dose
Table 4. Pretreatment Hemodynamic Variables in Anesthetized Dogs with CHF

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n=7)</th>
<th>Losartan (1 mg/kg)</th>
<th>E-3174 (0.3 mg/kg)</th>
<th>E-3174 (1 mg/kg)</th>
<th>Enalapril (0.3 mg/kg)</th>
<th>Enalapril (1 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>117±8.0</td>
<td>115±11.5</td>
<td>118±15.7</td>
<td>121±12.8</td>
<td>128±11.0</td>
<td>129±10.5</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>72±5.0</td>
<td>81±7.6</td>
<td>75±13.7</td>
<td>72±7.5</td>
<td>77±8.5</td>
<td>88±5.4</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>27.7±2.1</td>
<td>23.2±2.8</td>
<td>19.7±1.2</td>
<td>26.0±3.6</td>
<td>27.5±0.0</td>
<td>25.8±2.7</td>
</tr>
<tr>
<td>Pra (mmHg)</td>
<td>13.1±1.5</td>
<td>13.2±3.3</td>
<td>13.1±1.5</td>
<td>13.0±1.5</td>
<td>15.7±3.3</td>
<td>9.4±1.3</td>
</tr>
<tr>
<td>Ppa (mmHg)</td>
<td>43.4±3.7</td>
<td>36.6±4.1</td>
<td>40.0±0.0</td>
<td>38.4±3.2</td>
<td>48.0±1.7</td>
<td>39.0±7.2</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>28.0±3.2</td>
<td>23.8±2.5</td>
<td>27.0±3.0</td>
<td>27.2±2.5</td>
<td>31.0±2.1</td>
<td>24.6±2.7</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>9.0±1.3</td>
<td>11.4±2.0</td>
<td>10.3±1.0</td>
<td>8.6±0.9</td>
<td>7.1±1.3</td>
<td>10.4±1.0</td>
</tr>
<tr>
<td>TPR(mmHg/ml/min)</td>
<td>72.9±4.5</td>
<td>72.7±4.5</td>
<td>64.4±15.7</td>
<td>72.6±7.2</td>
<td>91.2±18.0</td>
<td>68.7±7.0</td>
</tr>
<tr>
<td>MFBF (ml/min)</td>
<td>41.1±9.3</td>
<td>33.0±7.4</td>
<td>42.3±18.4</td>
<td>28.1±7.7</td>
<td>44.3±13.7</td>
<td>61.6±7.0</td>
</tr>
<tr>
<td>MRBF (ml/min)</td>
<td>56.4±10.3</td>
<td>65.6±18.8</td>
<td>52.3±14.4</td>
<td>42.5±9.6</td>
<td>38.2±15.3</td>
<td>64.7±11.9</td>
</tr>
<tr>
<td>dP/dtP (s⁻¹)</td>
<td>21.7±3.2</td>
<td>33.8±4.2</td>
<td>27.3±4.0</td>
<td>22.3±2.8</td>
<td>20.3±4.0</td>
<td>27.2±3.0</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>46.9±6.1</td>
<td>35.8±2.1</td>
<td>35.6±2.3</td>
<td>40.2±2.2</td>
<td>44.1±4.1</td>
<td>39.6±1.5</td>
</tr>
</tbody>
</table>

n, number of animals; Pra, right atrial pressure; TPR, total peripheral resistance; MFBF, mean femoral blood flow; MRBF, mean renal blood flow; dP/dtP, peak first derivative of left ventricular pressure corrected for pressure; tau, left ventricular relaxation time constant. There were no significant differences in any pretreatment parameters among the groups (p>0.05, ANOVA).

Fig. 1. Effects of losartan (closed circles, 1 mg/kg), E-3174 (closed triangles, 1 mg/kg), enalapril (closed squares, 1 mg/kg), and vehicle (open circles) on mean arterial pressure (MAP). Values are the mean±SEM. * p<0.05 compared with vehicle at the corresponding time point.

values) and enalapril at 0.3 and 1 mg/kg (+45% and +104%, p<0.05). Losartan at 1 mg/kg also caused a significant increase of PRA (+74% from pre-dose values, p<0.05). There were no changes in PRA in dogs receiving vehicle. Therefore, the effects of 1 mg/kg of E-3174 on the RAS system were similar to the effects of 1 mg/kg of enalapril, while the effects of 1 mg/kg of losartan were slightly weaker.

Hemodynamic Changes after Drug Treatment

There were no significant differences among the six groups in baseline hemodynamic parameters (Table 4). E-3174 MAP immediately decreased after injection of E-3174 at 0.3 (−24±2%, p<0.05), and 1 mg/kg (−36±%, p<0.05) in a dose-dependent manner (Fig. 1), accompanied by a slight decrease in HR at 1 mg/kg (−15±%, p<0.05). MAP and HR gradually returned to baseline levels by 60 or 120 min after treatment. E-3174 at 0.3 and 1 mg/kg reduced LVEDP (−34±12% and −24±7%, p<0.05, Fig. 3), PCWP (−18±4% and −36±10%, p<0.05), and Ppa (−13±6% and −22±3%, p<0.05, Fig. 4). Pra was significantly decreased (−23±5%, p<0.05) by E-3174 at 1 mg/kg. The reductions in these pressures were accompanied by significant decreases in TPR (−23±5% and −41±9%, p<0.05) and PVR (−18±2% and −27±9%, p<0.05) at both doses, and a significant increase in SV at the dose of 1 mg/kg (+36±19%, p<0.05, Fig. 2). LV systolic wall stress was markedly reduced by both doses of E-3174 (p<0.05, Fig. 5). Intravenous E-3174 had no effect on the other variables, such as dP/dtP, tau, RBF, and FBF.

Losartan

The effects of losartan at 1 mg/kg on hemodynamics were similar to those obtained with 0.3 mg/kg of E-3174. Namely, immediately after treatment, reductions occurred in MAP (−21±5%, p<0.05, Fig. 1), HR (−7±3%, p<0.05), LVEDP (−21±5%, p<0.05, Fig. 3), PCWP (−13±7%, p>0.05), Pra (−10±3%, p<0.05) and Ppa (−13±6%, p<0.05, Fig. 4), then the values returned to baseline after 60 min. These reductions in pressures were accompanied by significant decreases in TPR (−23±6%, p<0.05) and PVR (−17±5%, p<0.05%) and a signifi-
Fig. 2. Effects of losartan (closed circles, 1 mg/kg), E-3174 (closed triangles, 1 mg/kg), enalapril (closed squares, 1 mg/kg), and vehicle (open circles) on stroke volume (SV). Values are the mean±SEM. *: p<0.05 compared with vehicle at the corresponding time point.

Fig. 3. Effects of losartan (closed circles, 1 mg/kg), E-3174 (closed triangles, 1 mg/kg), enalapril (closed squares, 1 mg/kg), and vehicle (open circles) on left ventricular end-diastolic pressure (LVEDP). Values are the mean±SEM. *: p<0.05 compared with vehicle at the corresponding time point.

Fig. 4. Effects of losartan (closed circles, 1 mg/kg), E-3174 (closed triangles, 1 mg/kg), enalapril (closed squares, 1 mg/kg), and vehicle (open circles) on pulmonary arterial pressure (Ppa). Values are the mean±SEM. *: p<0.05 compared with vehicle at the corresponding time point.

Fig. 5. Effects of losartan (1 mg/kg), E-3174 (1 mg/kg), enalapril (1 mg/kg), and vehicle on left ventricular systolic wall stress (WS). Values are the mean±SEM. *: p<0.05 compared with vehicle.

Significant increase in SV (+15±7%, p<0.05, Fig. 2). LV systolic wall stress was reduced by losartan, though it did not reach the level of statistical significance. Losartan had no meaningful effect on the other variables, such as dP/dt/P, tau, RBF and FBF.

Enalapril
Unlike rapid reduction in MAP after administration of E-3174 and losartan, intravenous enalapril at 0.3 and 1 mg/kg gradually reduced MAP (−40±9% and −39±3%, p<0.05, Fig. 1), HR (−11±2% and −13±4%, p<0.05), LVEDP (−43±11% and −32±5%, p<0.05, Fig. 3), PCWP (−31±3% and −34±6%, p<0.05), Ppa (−15±11% and −8±6%, p<0.05) and Ppa (−25±6% and −21±7%, p<0.05, Fig. 4) over 30 to 60 min after injection, and these changes persisted throughout the entire observation period. Both doses of enalapril lowered TPR (−34±10% and −35±2%, p<0.05) and PVR (−19±8% and −22±4%, p<0.05). Enalapril induced a transient and slight increase in SV at the dose of 1 mg/kg (+16±8%, p<0.05, Fig. 2). LV systolic wall stress was significantly reduced by both doses of enalapril (p<0.05, Fig. 5). Enalapril had no meaningful effect on the other variables, such as dP/dt/P, tau, RBF and FBF.

Echocardiographic Changes after Drug Administration
Echocardiographic variables before and 120 min after treatment in the six groups are presented in Table 5. There were no significant differences among the six groups in baseline values. None of the treatments caused any change in LVEDD.

E-3174 at 0.3 and 1 mg/kg caused a significant increase in FS (+47±8% and +56±8%, p<0.05, from baseline, respectively). Losartan at 1 mg/kg also increased FS (+52
Table 5. Effects of Losartan, E-3174 and Enalapril on Echocardiographic Measurements in Anesthetized Dogs with CHF

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Pre-injection</th>
<th>Post-injection (120 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS (%) Vehicle</td>
<td>7</td>
<td>10.7±2.0</td>
<td>11.4±1.3</td>
</tr>
<tr>
<td>Losartan (1 mg/kg)</td>
<td>4</td>
<td>12.4±2.0</td>
<td>18.3±1.5</td>
</tr>
<tr>
<td>E-3174 (1 mg/kg)</td>
<td>5</td>
<td>11.7±0.9</td>
<td>18.4±2.1*</td>
</tr>
<tr>
<td>Enalapril (1 mg/kg)</td>
<td>5</td>
<td>10.8±1.2</td>
<td>13.8±1.9</td>
</tr>
<tr>
<td>EDD (mm) Vehicle</td>
<td>7</td>
<td>41.8±2.0</td>
<td>40.9±2.5</td>
</tr>
<tr>
<td>Losartan (1 mg/kg)</td>
<td>4</td>
<td>40.7±1.7</td>
<td>38.9±2.3</td>
</tr>
<tr>
<td>E-3174 (1 mg/kg)</td>
<td>5</td>
<td>41.7±1.5</td>
<td>41.0±1.7</td>
</tr>
<tr>
<td>Enalapril (1 mg/kg)</td>
<td>5</td>
<td>38.3±1.6</td>
<td>38.4±1.6</td>
</tr>
</tbody>
</table>

FS, fractional shortening; EDD, left ventricular end-diastolic diameter. *p≤0.05 vs. before pacing (paired t-test). n: number of animals.

±12%, p<0.05). Enalapril at 0.3 and 1 mg/kg increased FS (+10±13% and +34±23%), though the changes at 1 mg/kg did not reach the level of statistical significance (p >0.05). The vehicle also had no effects on FS (+19±16%, p>0.05).

Discussion

E-3174, an active metabolite of losartan in humans, is considered to play a major role in the effects of losartan in heart failure as well as hypertension (19, 20). However, the hemodynamic effects of E-3174 on heart failure have not been sufficiently investigated experimentally. The present study provided the first demonstration that E-3174, an active metabolite of losartan in humans, reduced the pre- and after-load, resulting in improved SV in an experimental model of CHF in dogs with RRVP. When an acute treatment of the AT1 blockers is applied in a case of heart failure, the effective reduction of the pre- and after-load is the primary effect. An increase of SV, if observed, is considered to be a secondary effect.

RRVP is frequently used to produce CHF in dogs (2, 13, 14). The CHF model produced by RRVP is characterized morphologically by increased diastolic diameter of the ventricle with decreased wall thickness, and also by decreased cardiac output with or without increased total peripheral resistance. In the present study, the four-week period of rapid pacing caused severe symptoms of heart failure (ascites, cyanosis, decreased motor activity, decreased appetite, pulmonary edema and abnormal breathing) in dogs. These animals showed consequent elevation of the pre- and after-load of the left ventricle and decrease in CO. The absence of changes in HR in the present CHF model might reflect a reduction in baroreflex due to a continuous increase in sympathetic activity. In addition to the hemodynamic deterioration, these animals exhibited remarkable ventricular remodeling of LV dilatation characterized by increased LV inner diameter, thinning of the ventricular wall, and impairment of LV performance as demonstrated by a decrease in FS on echocardiography. These observations confirmed that our model represented a severe stage of heart failure (22, 23). The humoral factors in our model were also changed, as seen in several previous studies (1, 27-30). Namely, PRA, ALDO and NE were markedly elevated, as well as the plasma concentration of ANP.

It is known that losartan is rapidly metabolized to E-3174 after administration in humans (17, 18). The plasma concentration of E-3174 exceeds that of losartan within minutes and remains higher than that of losartan. Thus it is impossible to distinguish the effects of losartan from those of its metabolite, E-3174, in human subjects. In contrast to the situation in humans, losartan is hardly metabolized to E-3174 in dogs (16-18). The potency of E-3174 to block the AT1 receptor is over 30 times greater than that of losartan in vitro, and in vivo E-3174 is 15 times more potent than losartan in blocking pressor responses elicited by AII in normotensive rats (19). In addition to being more potent than losartan, the action of E-3174 is noncompetitive while that of losartan is competitive. A canine model is therefore appropriate for use in further characterizing the effects of losartan and E-3174 on CHF. The results of the present comparison in canines showed that there were no qualitative differences between the effects of losartan and those of E-3174.

In the present study, all three drugs improved the hemodynamics in experimental CHF. Both AT1 receptor antagonists and the ACEI reduced TPR. The AT1 receptor antagonists, losartan and E-3174, rapidly reduced MAP, and this effect reached a maximum within 10 min after administration of each drug. In contrast, the depressor responses to enalapril reached a plateau at 30-60 min after administration, and this plateau lasted up to 120 min, because enalapril is a produg and a certain time is required to form enalaprilat, the active metabolite. Enalapril is hydrolyzed to enalaprilat, an active metabolite of
enalapril that inhibits ACE activity and has vasodilatory activity with longer duration (26). It was demonstrated that complete conversion to enalaprilat in dogs occurred 30-60 min after intravenous administration of enalapril (26). Blockade of the AT1 receptor is expected to inhibit vasoconstriction by AII immediately after administration of a blocking agent. Furthermore, it has been suggested that, unlike enalapril, both E-3174 and losartan at therapeutic doses block thromboxane A2 receptors (30-32), which may account for some of the vasodilatory effects of these chemicals in addition to their blockade of the AT1 receptor. In addition, local AII in vascular beds may be formed not only by ACE but also by chymase (33). Therefore, together, these differences may account for the more rapid onset of vasodilatory effects with the AT1 blockers, losartan and E-3174, compared to the ACE inhibitor, enalapril.

A major difference between the AT1 receptor blockade and ACE inhibition involves the effect on stroke volume, since E-3174 was shown to result in a greater increase in SV than either enalapril or losartan. In this study, the acute hemodynamic effects of all three drugs were evaluated and there were no changes in dp/dtP or tau after administration of any of these compounds. These results suggested that none of these drugs exerted direct effects of contractile or relaxation performance in the ventricle when acutely administered. The decreases in heart rate were comparable after acute administration of these drugs.

In addition to reducing the formation of AII, ACEIs may also inhibit the degradation of bradykinin (26). A recent study suggested that inhibition of bradykinin degradation was involved in the improvement of cardiac function due to chronic ACEI administration in stroke-prone spontaneously hypertensive rats (34). Improvement of cardiac function by ramipril was reversed by concomitant administration of a BK2 receptor antagonist. However, the antihypertensive and antimitogenic effects of ramipril on the heart were not attenuated by blockade of the BK2 receptor. No study has examined the direct effects of acute or chronic bradykinin administration on cardiac function in CHF, and thus it is unclear whether elevation of endogenous bradykinin contributed to the acute hemodynamic effects of ACEI on CHF in the present study.

Because the present study was designed to determine the acute effects of AT1 receptor blockade or ACE inhibition, the differences in the improvement of cardiac performance may have resulted mainly from summation of the decreases in both pre- and after-load, that is, from the reduction in vascular resistance. If the increases in stroke volume were solely due to a reduction in vascular resistance, a more rapid reduction in vascular resistance would cause a prompt increase in stroke volume. On the other hand, the slow onset of vascular dilation seen after administration of enalapril may have been responsible for the fact that SV after enalapril administration was not significantly higher than that after administration of losartan or E-3174. This finding might also be attributed to differences in the changes of LVEDP; i.e., the reductions of LVEDP after administration of E-3174 at 1 mg/kg and losartan at 1 mg/kg were less than that after administration of enalapril. These changes reflected changes in the pre-load. We conclude that either of these compounds — but not enalapril — may cause a significant increase in SV.

A hemodynamic benefit of chronic treatment with ACEI in CHF dogs is an improvement in cardiac function that is reflected in an increase in cardiac output (8). AII is known to have not only a vasoconstrictive effect but also proliferative and mitogenic effects, and thus chronic blockade of RAS may also contribute to the long-term benefits of AII on cardiac performance (8). However, acute hemodynamic effects in established CHF are considered mainly to reflect vascular effects of ACEI and AT1 receptor blockade.

Although E-3174 and losartan did not demonstrate any apparent inotropic effects on hemodynamic measurements, the increase in FS determined by echocardiogram suggested that there were significant improvements in cardiac performance with both AT1 receptor antagonists. Administration of AII in dogs with CHF suppressed contraction and relaxation of the left ventricle by activating the AT1 receptor (35). In rats, AII exacerbated the impaired performance of the hypertrophied heart (36). Thus, the present finding that ACE inhibition by enalapril did not produce increases in SV and FS similar to those after AT1 receptor blockade may be partly due to incomplete blockading of local AII production by ACE-independent mechanisms; that is, the residual endogenous AII after ACE inhibition may have activated AT1 receptors in the failing heart and thereby further suppressed contraction and relaxation. Although indices for isovolumetric contraction were not affected by the treatments, FS reflected contractile forces not only in the isovolumetric period but also in the isotonic period (37). Therefore, blockade of the AT1 receptor may alter isotonic contraction without altering isovolumetric contraction. In addition, FS was estimated from cardiac movement at the LV cross-sectional view in the short-axis, and this method may not have been sufficient to reflect the total LV movement. Accordingly, more definitive studies will be needed to accurately evaluate the effects of the ejection phase.

In this study, PRA level was measured after administration of each of the three compounds, and the elevation of PRA after administration of 1 mg/kg of E-3174 was similar to that after 1 mg/kg of enalapril, while the increase in PRA after administration of 1 mg/kg of losartan was comparable to those after 0.3 mg/kg of E-3174 or enalapril. Since reflex elevation of PRA is considered to be an index of functional blockade of AII (5), the effect
of E-3174 on RAS is expected to be comparable to that of enalapril. In a previous study of tachycardia-induced CHF in dogs, captopril but not losartan increased cardiac output, accompanied by hypotension (5). These results were interpreted as suggesting that losartan may fail to block non-AT1 receptor-mediated AI1 activities adequately and captopril may exert its effects through non-AI1-mediated mechanisms. In the present study, a potent AT1 receptor antagonist, E-3174, increased SV at a dose of 1 mg/kg, elevating PRA to the same levels achieved by enalapril 1 mg/kg. Thus, the lack of improvement in cardiac output with losartan in the previous study may have been due to insufficient blockade of AT1 receptor rather than to lack of inhibition of AI1 action mediated by a non-AT1 receptor.

In conclusion, AT1 blockade as well as ACE inhibition can improve elevated loading conditions in the canine HF model, and the vasodilating effects of E-3174, an AT1 receptor blocker, are comparable to those of enalapril, an ACE inhibitor, and superior to those of losartan. The results demonstrated that E-3174, an active metabolite of losartan, plays a major role in the effects of losartan in heart failure. Thus, losartan therapy in patients with heart failure is expected to provide beneficial effects similar to those of enalapril with respect to elevated loading conditions.

References

22. Moe GW, Angus C, Howard RJ, Parker TG, Armstrong PW: Evaluation of indices of left ventricular contractility


