Increased endothelin-1 production in patients with chronic heart failure

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Parker, John D., and Jake J. Thiessen. Increased endothelin-1 production in patients with chronic heart failure. Am J Physiol Heart Circ Physiol 286: H1141–H1145, 2004; 10.1152/ajpheart.00239.2001.—Endothelin-1 (ET-1) concentrations are elevated in patients with congestive heart failure (CHF), although the cause of this increase remains uncertain. We hypothesized that abnormalities in ET-1 production, clearance, or a combination of these may be the cause of elevated ET-1 concentrations in chronic CHF. The kinetics of clearance of ET-1 were measured with125I-labeled ET-1 in eight patients with CHF and five age-matched normal individuals. In both normal subjects and the CHF group, the kinetics of ET-1 clearance were best described by a three-compartment model. The steady-state volume of distribution of ET-1 was significantly greater in the CHF group compared with normal subjects (25.2 ± 3.9 vs. 13.8 ± 2.1 l/kg; P < 0.05). The total clearance rate from plasma was greater in the CHF group (0.119 ± 0.02 vs. 0.047 ± 0.013 l/kg·min−1; P < 0.05). It appears that increased ET-1 production rather than decreased clearance is the cause of elevated ET-1 concentrations in patients with chronic CHF.

of patients with severe CHF as well as a group of age-matched volunteers. Our intention was to determine the kinetics of clearance and production of ET-1 in the setting of chronic CHF in an effort to determine the etiology of elevated circulating levels of ET-1 in CHF. We hypothesized that abnormalities in ET-1 production, clearance, or a combination of these may be the cause of elevated ET-1 concentrations in chronic CHF.

METHODS

Subjects. Eight patients (6 men, 2 women) with heart failure were studied. Inclusion criteria included New York Heart Association class 3–4 functional status and a radionuclide ejection fraction of <25%. Patients were excluded if they had experienced an unstable ischemic syndrome within the last 6 mo. Details concerning concomitant diseases and medical therapy in these patients are presented in Table 1. Five healthy male volunteers, matched in age with the CHF group, were recruited through advertisements. All individuals underwent a screening history and physical examination by a cardiologist. The protocol was approved by the Human Subjects Review Committee of the University of Toronto, and written informed consent was obtained in all cases.

Study protocol. Studies were performed in the morning beginning at 8 AM. Subjects were requested to fast overnight and to refrain from vigorous exercise and ethanol ingestion for 24 h before the study. On arrival, a standard 18-gauge intravenous line was inserted into the dominant forearm. A 3.0-Fr, 20-gauge arterial catheter (Cook, Bloomington, IN) was inserted into the radial artery of the nondominant arm for blood pressure monitoring and blood sampling. For measurement of the right atrial pressure, a 5.0-Fr, 10-cm sheath (Terumo Medical, Elkton, MD) was inserted into the brachial vein, through which a 19-gauge, 61-cm Intracath Vialon IV catheter (Becton Dickinson, Sandy, UT) was passed until a right atrial pressure waveform was obtained. The intrathoracic position of the catheter was confirmed by the presence of typical respiratory variation in the pressure waveform. Catheters were placed under local anesthesia without sedation. Pressure recordings were obtained through a Perceptor Morse Manifold pressure transducer (Namic, Glens Falls, NY), ECG, blood pressure, and right atrial pressure recordings were made on a strip chart recorder (model 210-180, Mennen Medical, St. Clarence, NY). Individual models of CHF, treatment with ET-1 antagonists has been shown to have beneficial effects (4, 17, 28, 29). At present, it remains unclear whether the increase in plasma concentration of ET-1 observed in CHF is secondary to increases in production and/or clearance of this peptide in CHF.

Recently, we reported (24) the clearance kinetics of ET-1 with an125I-labeled ET-1 (125I-ET-1) ratiodrator infusion technique in a group of young, normal volunteers. This approach demonstrated that the kinetics of clearance of ET-1 is complex, with three-compartment characteristics, a wide volume of distribution, and prolonged terminal clearance. In the present study we examined the kinetics of clearance of ET-1 in a group

ENDOTHELIN-1 (ET-1) is a 21-amino acid protein that has potent and prolonged vasoconstrictor properties (22, 33). ET-1 receptors have been described in a variety of organ systems. Although the autocrine and paracrine activity of ET-1 is increasingly recognized, circulating ET-1 in humans appears to be of pathophysiological relevance (1, 7, 20, 27, 30). This is underscored by the presence of increased plasma levels in a variety of cardiovascular disease states (7, 20, 27, 30). Importantly, infusions of exogenous ET-1 to achieve circulating levels similar to those seen in pathophysiological states have resulted in significant and hemodynamic effects (19). In the setting of congestive heart failure (CHF), elevated ET-1 levels correlate with disease severity and the presence of pulmonary hypertension and are predictive of poor prognosis (7, 26). In animal models of CHF, treatment with ET-1 antagonists has been shown to have beneficial effects (4, 17, 28, 29). At present, it remains unclear whether the increase in plasma concentration of ET-1 observed in CHF is secondary to increases in production and/or clearance of this peptide in CHF.

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- Measurements of plasma ET-1 and 125I-labeled ET-1. Measurements of ET-1 and 125I-ET-1 were carried out with radi免疫assay and scintillation counting after passage through a silica C18 cartridge (Sep-Pak, Waters) as described in our previous report (24). The recovery of 125I-ET-1 spiked into a plasma pool was 88.2 ± 11.3%. Methods to confirm the identity of the Sep-Pak-extracted radioactive residue as ET-1 were also reported previously (24).

- Pharmacokinetic analysis. The plasma 125I count concentrations were analyzed by means of multiexponential equations with techniques we described previously (24). The analysis allowed for the characterization of the multicompartment nature of ET-1 clearance kinetics and the calculation of initial (V(1)) and steady-state (V(s)) distribution volumes as well as the calculation of ET-1 elimination half-lives (see Table 4). The use of a radiolabeled tracer allows for the calculation of total body clearance (Cl(t)) and production rates of endogenous compounds. A traditional approach to this problem is the method described by Esler et al. (12), in which the kinetics of norepinephrine are determined with a constant infusion of tritium-labeled norepinephrine given to achieve steady state. For example, Cl(t) can be defined as the infusion rate of labeled norepinephrine divided by the concentration of the tracer in plasma at steady state. When the terminal half-life of the concentration vs. time curve is comparatively long, as seen after 125I-ET-1, it becomes impractical to use the constant-infusion approach because the time to achieve steady-state concentration is too long. Nevertheless, analysis of 125I count vs. time concentration curves after the bolus administration of 125I-ET-1 does allow for calculation of whole body ET-1 clearance and production rates. When using this approach, it is important to capture as much of the area under the curve (AUC) as possible, by using a sampling schedule that encompasses a significant portion of the period of distribution equilibrium (V(s)). With this approach, Cl(t) of ET-1 can be calculated as dose of 125I-ET-1/AUC, where AUC is derived from the observation period (0–240 min). Subsequently, total body ET-1 production can be estimated as Cl(t) × endogenous ET-1 concentration, where the endogenous ET-1 concentration is the average of ET-1 concentrations during the period of distribution equilibrium (60–240 min).

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CHF</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>58±4</td>
<td>56±3</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>174±3</td>
<td>171.5±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76±8</td>
<td>73±5</td>
<td>NS</td>
</tr>
<tr>
<td>NYHA class</td>
<td>3.2±0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>13±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic cardiomyopathy, %</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors, %</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diuretics, %</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digoxin, %</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blockers, %</td>
<td>60</td>
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<td></td>
</tr>
</tbody>
</table>

CHF, congestive heart failure; NYHA class, New York Heart Association functional class; ACE, angiotensin-converting enzyme; NS, not significant.

### Table 2. Hemodynamics

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Normal</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR, beats/min</td>
<td>SBP, mmHg</td>
</tr>
<tr>
<td>0</td>
<td>65±4</td>
<td>129±8</td>
</tr>
<tr>
<td>10</td>
<td>67±6</td>
<td>134±7</td>
</tr>
<tr>
<td>30</td>
<td>65±3</td>
<td>130±8</td>
</tr>
<tr>
<td>240</td>
<td>67±3</td>
<td>135±7</td>
</tr>
</tbody>
</table>

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; RAP, right atrial pressure. Time is from start of 125I-labeled endothelin-1 infusion. *P < 0.05 vs. normal group, 0 min.

### Table 3. Plasma endothelin-1 measurements

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Normal, pg/ml</th>
<th>CHF, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.10±0.10</td>
<td>2.10±0.29*</td>
</tr>
<tr>
<td>5</td>
<td>1.28±0.07</td>
<td>2.25±0.50</td>
</tr>
<tr>
<td>15</td>
<td>1.32±0.08</td>
<td>1.86±0.32</td>
</tr>
<tr>
<td>60</td>
<td>1.32±0.11</td>
<td>1.99±0.45</td>
</tr>
<tr>
<td>240</td>
<td>1.24±0.09</td>
<td>1.73±0.26</td>
</tr>
</tbody>
</table>

Time is from start of 125I-labeled endothelin-1 infusion. *P < 0.05 vs. normal group, 0 min.

### Statistical analysis

Within-group comparisons of the effects of 125I-ET-1 infusion on hemodynamics and circulating ET-1 levels were made with analysis of variance. Between-group comparisons of baseline hemodynamics and circulating ET-1 levels were made with an unpaired t-test. ET-1 kinetic parameters were often not normally distributed, and comparison of these parameters was made with the Wilcoxon-Mann-Whitney rank sum test. Data were analyzed with Statview 5.0 (SAS Institute Software, San Rafael, CA). Data are presented as means ± SE. For clearance kinetics variables, mean and median values are presented. A P value of <0.05 was required for statistical significance.

### RESULTS

#### Hemodynamic effects of 125I-ET-1 infusion

Although hemodynamics were recorded at all time points, only data obtained at 0, 10, 30, and 240 min hemodynamics are presented (Table 2). In the CHF group, resting heart rate and right atrial pressure were higher and diastolic arterial pressure was lower compared with the normal group (P < 0.05). 125I-ET-1 infusion caused no change in any hemodynamic parameter in either group.

**Plasma ET-1 level.** Plasma ET-1 levels measured at 0, 5, 15, 60, and 240 min are presented in Table 3. At baseline (time 0) arterial ET-1 concentrations were significantly higher in the CHF group than in the age-matched normal group (2.1 ± 0.29 pg/ml vs. 1.7 ± 0.1 pg/ml; P = 0.021). There was no significant change in ET-1 levels after 125I-ET-1 infusion in either group.

**ET-1 kinetics.** The composite plasma 125I-ET-1 counts concentration vs. time profiles for both normal volunteers and CHF patients are presented in Fig. 1. There was an initial rapid rise in counts during the 5-min infusion. On completion of the infusion, counts fell rapidly initially and then much more gradually over time. The plasma concentration vs. time profiles were not described by a single exponential disappearance profile, and it appeared that a multieponential function best described the data. With the combined data for each group, computer fitting was used to identify the most appropriate relationship. A biexponential function, reflecting a two-compartment model, appeared to be unsatisfactory. The nearest...
neighbor residual test indicated a systematic, statistically significant \( P < 0.01 \) deviation that pointed to the requirement for an additional exponential. A triexponential function, associated with a three-compartment pharmacokinetic model, was the simplest mathematical expression that adequately described the data set for both normal subjects and CHF patients. The data from each case in both groups were then individually fit with both the biexponential and triexponential functions. In all cases, a triexponential function provided the best fit. Detailed pharmacokinetic parameters for the three-compartment model are presented in Table 4.

A comparison of normal subjects and CHF patients showed that the \( V_{ss} \) of ET-1, although large in both groups, was significantly greater in the CHF group compared with normal subjects \((25.2 \pm 3.9 \text{ vs. } 13.8 \pm 2.1 \text{ l/kg}; \, P < 0.05; \, \text{Table 4})\). The total clearance rate from plasma was greater in the CHF group compared with the normal subjects \((0.119 \pm 0.018 \text{ vs. } 0.047 \pm 0.013 \text{ l/kg}^{-1}\text{-min}^{-1}; \, P = 0.05; \, \text{Table 4})\). As in our previous study, the terminal half-life of ET-1 (\( \gamma \) half-life in triexponential function) in both groups was much larger than previously reported. Although the terminal half-life in the CHF group was quite variable, it was longer than that observed in the normal group \((P = 0.04; \, \text{Table 4})\). The total body production rate of ET-1 was significantly higher in patients with CHF than in the age-matched normal group \((0.21 \pm 0.03 \text{ vs. } 0.06 \pm 0.02 \text{ ng-kg}^{-1}\text{-min}^{-1}; \, P < 0.05; \, \text{Table 4})\).

There was a significant correlation between the ET-1 production rate and the mean ET-1 concentration rate during the period of distribution equilibrium \((60–240 \text{ min after the time of administration}; \, R = 0.73, \, P < 0.001; \, \text{Fig. 2})\). There was no significant correlation between ET-1 concentration and the ET-1 clearance rate \((R = 0.04, \, P = \text{not significant})\).
three-compartment model, a wide volume of distribution, and a very long terminal half-life. With the present study, we extend these observations to a group of patients with chronic CHF, contrasting the findings in this group to those in a group of subjects, similar in age, with normal cardiovascular physiology. Our findings demonstrated important changes in ET-1 kinetics in the setting of chronic heart failure. Our results emphasize that increased ET-1 levels in CHF are not the result of decreased clearance but rather reflect increase in production. Recent investigations suggest that ET-1 antagonists may have therapeutic value in CHF (4, 17, 18, 28, 29). Because both nonselective and selective ET\(_A\) receptor antagonists could have an impact on ET-1 production and clearance, an improved understanding of the kinetics of this peptide will be helpful in understanding the effect of these drugs in CHF.

As in our previous report (24), we find that a three-compartment model best describes the kinetics of clearance of ET-1. The plasma associated with the sampling compartment (compartment 1) is part of the early phase. This is also seen in the relative magnitude of the initial distribution space (V\(_I\); Table 4). Compartments 2 and 3 reflect slower or more delayed equilibration. They contribute strongly to the estimates of steady-state distribution space (V\(_ss\)), ET-1 has a very large V\(_ss\), demonstrating that ET-1 is taken up extensively into cells throughout the body, such that relatively little peptide is found in the initial zone that includes the plasma. It is likely that the large observed volume of distribution represents binding and internalization of ET-1 into various organ systems (5, 9, 14). The CHF group had a significantly larger V\(_ss\), suggesting greater distribution of ET-1, an observation consistent with the finding of increased tissue ET-1 concentrations in a number of organs in the setting of CHF (13, 23, 25, 34).

The total plasma clearance of ET-1 is high, averaging 47 ml\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) in the normal group and 119 ml\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\) in the CHF group. Because of a high degree of tissue uptake, as defined by V\(_ss\), the terminal half-life is comparatively long. The CHF patients were found to have a higher rate of clearance. Despite this, the CHF group displayed a significantly longer terminal half-life. Although these two parameters might seem to be inconsistent with each other, the larger volume of distribution in the CHF patients explains the long terminal half-life despite the presence of a higher total clearance rate.

Data from the present study demonstrate that both ET-1 production and clearance are increased in chronic CHF. Therefore, the increased plasma ET-1 concentrations observed in CHF are not secondary to abnormalities in clearance but are secondary to increased production. Indeed, the finding that clearance and production are increased in the setting of CHF suggests that clearance mechanisms play an important role in minimizing the increase in ET-1 plasma concentrations observed in CHF. It is important to emphasize that only total body kinetics were measured in this study. Therefore, no comment can be made about the site(s) of increased ET-1 production in patients with CHF. A number of studies, both animal and human, have suggested that ET-1 production is increased in a number of tissues, and it is possible that several organ systems contribute to the observed increase in ET-1 production. Similarly, the mechanism of increased ET-1 clearance in CHF is uncertain. In the setting of normal physiology, the lungs are a very important site of ET-1 clearance, with transpulmonary extraction of ET-1 reported to be in excess of
disease states. Application of this technique may lend important insights into the mechanisms of elevations in ET-1 levels in other pathological states.

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REFERENCES