Increased endothelin-1 production in patients with chronic heart failure

John D. Parker and Jake J. Thiessen

Division of Cardiology, Department of Medicine, and Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada M5G 1X5

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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.—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 with 125I-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 ± 0.018 vs. 0.047 ± 0.013 L·min⁻¹·kg⁻¹; P = 0.05). The total body production rate of ET-1 was also significantly higher in patients with CHF (0.21 ± 0.03 vs. 0.06 ± 0.02 ng·kg⁻¹·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.

METHODOLOGY

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 Perceptron 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). Individuals rested in a supine position in a quiet, comfortable environment for 45 min after the catheters were placed. To ensure hemodynamic stability, blood pressure, right atrial pressure, and heart rate recordings were made every 15 min.

125I-ET-1 infusion. Fifty microcuries (64 ng) of 125I-ET-1 (New England Nuclear, Wilmington, DE) were mixed in 50 ml of 5% dextrose in water and infused through the peripheral intravenous line at a rate of 10 ml/min for 5 min with a Harvard 33 Pump (Harvard Apparatus, St. Laurent, Quebec, Canada). Heart rate, blood pressure, right atrial pressure, and arterial blood samples for counting of 125I and measurement of ET-1 were obtained at −15 min and 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 100, 120, 150, 180, 210, and 240 min after the start of the infusion.

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). Importantly, infusions of exogenous ET-1 to achieve circulating levels similar to those seen in pathological 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.

Recently, we reported (24) the clearance kinetics of ET-1 with an 125I-labeled ET-1 (125I-ET-1) radiotracer 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 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.

Address for reprint requests and other correspondence: J. D. Parker, Division of Cardiology, Dept. of Medicine, Mount Sinai Hospital, Suite 1609, 600 Univ. Ave., Toronto, Ontario, Canada MSG IX5 (E-mail: jdp@ca.inter.net).

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Measurement of plasma ET-1 and $^{125}$I-labeled ET-1. Measurements of ET-1 and $^{125}$I-ET-1 were carried out with radiomunnoassay and scintillation counting after passage through a silica C18 cartridge (Sep-Pak, Waters) as described in our previous report (24). The recovery of $^{125}$I-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 $^{125}$I count concentrations were analyzed by means of multieponential 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 ($V_I$) and steady-state ($V_{ss}$) 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 ET-1 production can be estimated as $C_{lt} \times$ 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).

Statistical analysis. Within-group comparisons of the effects of $^{125}$I-ET-1 infusion on hemodynamics and circulating ET-I levels were made with analysis of variance. Between-group comparisons of baseline hemodynamics and circulating ET-I 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 the 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 $^{125}$I-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 ($P < 0.05$). $^{125}$I-ET-1 infusion caused no change in any hemodynamic parameter in either group.

Pharmacokinetic analysis. The composite plasma $^{125}$I-ET-1 counts concentration vs. time profiles for both normal volunteers and CHF patients are presented in Table 3. At baseline (time 0) arterial ET-I concentrations were significantly higher in the CHF group than in the age-matched normal group (2.1 ± 0.29 vs. 1.1 ± 0.1 pg/ml; $P = 0.021$). There was no significant change in ET-1 levels after $^{125}$I-ET-1 infusion in either group.

ET-1 kinetics. The composite plasma $^{125}$I-ET-1 counts vs. time profiles for both normal volunteers and CHF patients are presented in Table 3. At baseline (time 0) arterial ET-I concentrations were significantly higher in the CHF group than in the age-matched normal group (2.1 ± 0.29 vs. 1.1 ± 0.1 pg/ml; $P = 0.021$). There was no significant change in ET-1 levels after $^{125}$I-ET-1 infusion in either group.
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. 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 ± 3.9 vs. 13.8 ± 2.1 l/kg; \( P < 0.05 \); Table 4). The total clearance rate from plasma was greater in the CHF group compared with the normal subjects (0.119 ± 0.018 vs. 0.047 ± 0.013 l/kg\(^{-1}\)-min\(^{-1} \); \( P = 0.05 \); 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 (\( P = 0.04 \); 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 ± 0.03 vs. 0.06 ± 0.02 ng\( \cdot \)kg\(^{-1}\)\(-\)min\(^{-1} \); \( P < 0.05 \); 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 min after the time of administration; \( R = 0.73, P < 0.001 \); Fig. 2). There was no significant correlation between ET-1 concentration and the ET-1 clearance rate \( [R = 0.04, P = \text{not significant}] \).

**DISCUSSION**

Previously, we (24) reported the clearance kinetics of ET-1 in a group of young, normal men, documenting that the clearance kinetics of ET-1 are complex, characterized by a

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**Table 4. Summary of pharmacokinetic parameters from triexponential (three-compartment model) fit of individual data sets**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal</th>
<th>CHF</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_i ), l/kg</td>
<td>0.4 [0.4]</td>
<td>0.6 [0.4]</td>
<td>NS</td>
</tr>
<tr>
<td>( V_{ss} ), l/kg</td>
<td>13.8 [13.9]</td>
<td>25.2 [25.6]</td>
<td>0.05</td>
</tr>
<tr>
<td>( \alpha_{t_{1/2}} ), min</td>
<td>(2.1)</td>
<td>(3.9)</td>
<td>NS</td>
</tr>
<tr>
<td>( \beta_{t_{1/2}} ), min</td>
<td>(0.17)</td>
<td>(0.27)</td>
<td>NS</td>
</tr>
<tr>
<td>( \gamma_{t_{1/2}} ), min</td>
<td>(5.0)</td>
<td>(5.0)</td>
<td>NS</td>
</tr>
<tr>
<td>( \alpha_{\min^{-1}} ), min(^{-1} )</td>
<td>(0.17)</td>
<td>(0.13)</td>
<td>NS</td>
</tr>
<tr>
<td>( \beta_{\min^{-1}} ), min(^{-1} )</td>
<td>(0.007)</td>
<td>(0.009)</td>
<td>NS</td>
</tr>
<tr>
<td>( \gamma_{\min^{-1}} ), min(^{-1} )</td>
<td>(0.0004)</td>
<td>(0.0002)</td>
<td>0.08</td>
</tr>
<tr>
<td>Total body clearance, kg(^{-1})-min(^{-1} )</td>
<td>0.047 [0.035]</td>
<td>0.119 [0.107]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total body production, ng( \cdot )kg(^{-1})(-)min(^{-1} )</td>
<td>0.06 [0.05]</td>
<td>0.21 [0.18]</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Values are means (SE in parentheses, medians in brackets). \( V_i \), initial volume of distribution; \( V_{ss} \), volume of distribution at steady state; \( t_{1/2} \), half-life of a given exponential; \( \alpha, \beta, \gamma \), 3 constants in the triexponential function; clearance, total clearance from plasma; production, total presentation to plasma.

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![Graph](http://ajpheart.org/)

**Fig. 2. Relationship between the endothelin-1 (ET-1) production rate and the mean ET-1 concentration rate during the period of distribution equilibrium (60–240 min after the time of administration).**

\( Y = 0.91 + 4.798 \times X_1 \); \( R = 0.727 \)
The CHF patients were found to have a higher rate of clearance of ET-1 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 increased production. Recent investigations suggest that ET-1 antagonists may have therapeutic value in CHF (4, 17, 18, 28, 29). Because both nonselective and selective ETA 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 (V1; Table 4). Compartments 2 and 3 reflect slower or more delayed equilibration. They contribute strongly to the estimates of steady-state distribution space Vss. ET-1 has a very large Vss, demonstrating that ET-1 is taken up extensively into cells throughout the body, such that relatively little peptide is found in the initial phase 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 Vss, 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·kg⁻¹·min⁻¹ in the normal group and 119 ml·kg⁻¹·min⁻¹ in the CHF group. Because of a high degree of tissue uptake, clearance is very long, as defined by Vss, 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 45% (10). In humans with pulmonary hypertension secondary to left ventricular dysfunction and mitral stenosis, pulmonary clearance of ET-1 was reduced (8). Similarly, in rats with heart failure secondary to myocardial infarction, pulmonary clearance of ET-1 was reduced (11). These studies used the bolus indicator dilution technique to determine transpulmonary kinetics. Importantly, this technique does not provide measurements of total body ET-1 clearance. Furthermore, this approach measures ET-1 transorgan extraction immediately after bolus injection of labeled ET-1, providing a measure of ET-1 uptake during the early distribution phase of the kinetics profile. These results may not be representative of ET-1 clearance under steady-state conditions. Of note, recent publications documented net extraction of ET-1 when arterial and venous ET-1 concentrations were measured across the heart (27) and the lower extremity in patients with severe CHF (27, 28). This observation is consistent with our kinetics-based observation that ET-1 clearance is increased in CHF. In contrast, one of these reports described significant transpulmonary production of ET-1 in those with severe CHF (27). Together, these observations serve to emphasize that ET-1 kinetics are abnormal in CHF and that these abnormalities may be organ specific.

Some limitations of our study should be considered. The patients with heart failure in this study were treated with a number of medications, some of which could have an impact on ET-1 production and clearance. In particular, angiotensin I-converting enzyme (ACE) inhibition was used in all of the heart failure patients. In recent studies, the administration of an ACE inhibitor was reported to cause a significant reduction in endogenous ET-1 levels in patients with CHF (15, 32). Those authors suggested that this decrease in endogenous ET-1 levels was caused by a decrease in ET-1 production, citing previous evidence that angiotensin II plays an important role in the promotion of ET-1 production (3, 6, 21). Of note, some studies failed to confirm an effect of ACE inhibition on endogenous ET-1 levels (16, 31). Our results demonstrate that ET-1 production rates continue to be elevated in patients with CHF despite treatment with an ACE inhibitor. We have used a radiotracer infusion technique in which 125I-ET-1 was infused as opposed to synthetic unlabeled ET-1. 125I is incorporated at the Tyr13 site of the ET-1 molecule. Although it is possible that substitution of 125I for a hydrogen molecule normally present at that site may alter the stereochemistry of the molecule, proprietary data from New England Nuclear as well as a study by Anggard et al. (2) have shown that the labeled and unlabeled species display the same chromatographic mobilities on HPLC. 125I-ET-1 has the same biological activity and displays similar binding affinities for antibody sites. In addition, unlabeled ET-1 is able to displace 125I-ET-1 from ET-1-specific antisera in similar concentrations, all of which suggests that using 125I-ET-1 to determine the kinetics of endogenous ET-1 is reasonable.

In conclusion, this study demonstrates that in humans ET-1 has a large volume of distribution, a prolonged terminal half-life in plasma, and a pharmacokinetic profile most consistent with a three-compartment model of clearance as determined by an infusion of radiiodinated ET-1. In CHF, ET-1 pharmacokinetics are different, with greater peripheral uptake, greater total body production, and increased total clearance rates. This study demonstrates that use of radiotracer techniques can differentiate normal ET-1 kinetics from those observed in
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... 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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