Reduced Pulmonary Clearance of Endothelin-1 Contributes to the Increase of Circulating Levels in Heart Failure Secondary to Myocardial Infarction

Jocelyn Dupuis, MD, PhD, FRCP; Jean-L. Rouleau, MD; Peter Cernacek, MD

Background—The pulmonary vascular bed is a major site for endothelin-1 (ET-1) clearance. A reduced clearance could contribute to the increase in circulating ET-1 levels found in heart failure (HF). We therefore evaluated the effect of HF on pulmonary ET-1 clearance and on plasma ET-1 concentrations.

Methods and Results—Rats with myocardial infarction (n=24) were compared with sham-operated rats (n=22). The lungs were isolated and perfused at a constant flow rate of 10 mL/min. Pulmonary ET-1 clearance was measured by the single-bolus indicator-dilution technique with 125I-labeled ET-1. Infarct rats developed HF with mild pulmonary hypertension. ET-1 extraction was reduced by HF from 63±1.5% to 41±4.5% (mean±SEM, P<0.001). Mixed venous (MV) and aortic ET-1 levels doubled with HF. There was a plasma ET-1 gradient across the lungs of sham rats (MV–aortic levels, 0.21±0.12 pg/mL) but not in lungs of HF rats (0.01±0.17 pg/mL). Plasma ET-1 levels correlated closely and inversely with ET-1 extraction (P<0.001).

Conclusions—HF is associated with reduced pulmonary ET-1 clearance that contributes to the increase in circulating levels. (Circulation. 1998;98:1684-1687.)

Key Words: endothelin ■ heart failure ■ pulmonary heart disease ■ myocardial infarction ■ lung

Heart failure (HF) is associated with increased circulating endothelin-1 (ET-1) levels that correlate with the severity of this condition.1–3 The increase of circulating ET-1 levels has been found to correlate closely with the degree of pulmonary hypertension.4 In dogs, ET-1 infusion that reproduces the concentrations found in HF causes significant hemodynamic effects.5 Physiologically, ET-1 is a paracrine/autocrine factor, but its elevated concentrations in HF suggest that ET-1 may also contribute to the counterregulatory neurohumoral activation.

The causes of the increase in circulating ET-1 levels in HF are unknown. The pulmonary vascular bed is an important site for both clearance and production of ET-1.6,7 In rats, the lung is the major site for ET-1 clearance.8 In normal humans, the lung clears ∼50% of circulating ET-1 in mixed venous (MV) blood and releases into the circulation a quantitatively similar amount, such that there is no or a very mild negative arteriovenous difference of ET-1 levels. In human pulmonary hypertension of various causes, systemic arterial ET-1 levels become equal to or even slightly higher than venous levels.9 Similarly, ET-1 in blood taken from a pulmonary artery catheter advanced to the capillary wedge position in patients with HF is increased compared with pulmonary artery levels,10 also suggesting that the pulmonary circulation may contribute to this increase through a reduced clearance, an increase in production, or a combination of both.

Although pulmonary clearance of ET-1 has been confirmed in various mammals, including humans, the possible contribution of a reduced clearance to the increase in circulating ET-1 has never been evaluated. The aim of the present study was therefore to evaluate the effect of HF on pulmonary ET-1 clearance and circulating ET-1 levels.

Methods

Myocardial Infarction

Male Wistar rats (Charles River, St-Constant, Quebec, Canada) 7 weeks old and weighing 200 to 250 g were used. Animals were given water and rat chow ad libitum and subjected to 12-hour light/dark cycles. The investigations performed conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1985). The animals were anesthetized with 1% halothane in a mixture of 100% O2. A left lateral thoracotomy was performed, and the heart was rapidly exteriorized, the left anterior descending coronary artery ligated, and the heart replaced in the thorax. The chest was closed with a Mikron wound clip applicator (Clay Adams) after the chest was gently pressed to expel air from the pleural cavity. The animals were allowed 48 hours to recover, during which time there was an initial mortality of ∼40%. At 48 hours, an ECG was performed, and the criteria described by Pfeffer et al11 were used to evaluate infarct size. Rats with ECG criteria of moderate to large myocardial infarctions were kept for study (n=24), and those with little or no evidence of infarction were discarded. Sham-operated rats (n=22) underwent the same surgical procedure except for coronary artery ligation.
In Vivo Hemodynamics and Lung Isolation

Four weeks after surgery, the animals were again anesthetized with halothane followed by 2000 U heparin IP (Sigma Chemical Co). After stable anesthesia was attained, the right jugular vein and left carotid artery were isolated, and fluid-filled catheters (PE50) were inserted for measurements of right ventricular and left ventricular pressures and dP/dt. A 1.5-mL sample of blood was then taken simultaneously from the aorta and the right ventricle for measurement of plasma immunoreactive ET-1 as previously described. The collection time of 28 seconds.

The effluent in a linear fraction collector containing 30 glass tubes with proximal to the lungs and simultaneous collection of the lung perfusate was performed to observe the heart and lungs, and the pulmonary artery was cannulated through an incision in the right ventricle. Another cannula was inserted in the left atrium through an incision in the left ventricle for collection of the lung effluent. Lung perfusion was initiated at 2.0 mL/min with Krebs solution containing (mmol/L) NaCl 120, NaHCO3 25, KCl 4.7, KH2PO4 1.18, MgSO4 1.17, CaCl2 2.5, and glucose 5.5. The Krebs solution was bubbled with 95% O2/5% CO2 to maintain a pH of 7.4. The lungs and heart were then rapidly isolated and suspended in a warm (37°C) water-jacketed chamber, in which the lungs were perfused in nonrecirculating fashion with Krebs solution supplemented with 3% albumin. The pulmonary flow was continuously measured with a transonic flow probe (Transonic) connected to a flowmeter (model 208) proximal to the pulmonary cannula. The perfusion pressure was continuously recorded by a fluid-filled pressure transducer connected to a Gould signal conditioner.

The lungs were then perfused under constant flow conditions for 20 minutes of stabilization with a Masterflex roller pump (Cole-Palmer) at a rate of 10 mL/min. Single-bolus indicator-dilution experiments were carried out at the end of each equilibration period by injection of a bolus in the perfusion cannula immediately proximal to the lungs and simultaneous collection of the lung effluent in a linear fraction collector containing 30 glass tubes with a collection time of 28 seconds.

Pulmonary ET-1 Extraction

The indicator-dilution technique was used to quantify the pulmonary metabolism of ET-1 as previously described. Briefly, a bolus containing trace doses of 125I-labeled ET-1 and a nonmetabolizable vascular reference (Evans blue dye bound to albumin) is injected into the pulmonary circulation, and timed sequential outflow samples are collected. The quantity of both tracers in each of the collected samples is determined and normalized to the total amount of activity injected to obtain the fractional recovery of each tracer per milliliter of lung effluent. The fractional recoveries can then be plotted as a function of time to construct the indicator-dilution curve. The tubes containing the lung effluent were processed by addition of 2.0 mL 0.9% saline to each and vortexing. A 1-mL aliquot from each tube was placed in a gamma counter to measure 125I activity, and in another 1-mL aliquot, Evans blue dye absorbance (620–740 nm) was measured. The fractional recovery of each tracer in each sample was then determined, and the indicator-dilution curves were constructed as above.

Heart Morphometric Analysis

After completion of the indicator dilution studies, the weights of the right ventricle and of the left ventricle plus septum were determined. The scar from the left ventricle of the infarct group was then excised and weighed and its surface area measured by planimetry.

Statistical Analysis

Differences between the sham and infarct rats were analyzed by 2-tailed unpaired t test. Differences between aortic and MV ET-1 levels within each group were analyzed by 2-tailed paired t test. A value of P<0.05 was considered significant. All values are reported as mean±SEM.

Results

Study parameters obtained from the 2 groups are assembled in the Table. At the end of 4 weeks, there was no difference in body weight between the 2 groups. Rats in the HF group had infarcts of medium size as assessed from scar weight and surface area. Compared with the sham group (n=22), the HF group (n=24) developed lower mean arterial pressure and left ventricular dP/dt and higher left ventricular end-diastolic pressure. The HF rats also developed significant pulmonary hypertension, as evidenced by higher right ventricular systolic pressure, but did not develop right ventricular hypertrophy. MV and aortic ET concentrations were obtained in 22 rats from the sham group and 17 rats from the HF group. Both aortic and MV ET-1 levels almost doubled with HF. Although aortic levels were lower than MV levels in the control group, they were not different in the HF group. Pulmonary ET-1 gradients across the lungs were computed as the differences between MV minus aortic ET-1 levels (Figure 1). The mean gradient was positive in the sham rats at 0.21±0.12 pg/mL but was close to zero in HF rats at 0.01±0.17 pg/mL.

Study Parameters for the Two Groups

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=22)</th>
<th>HF (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>399±20</td>
<td>394±11</td>
</tr>
<tr>
<td>Infarct weight, mg/g body wt</td>
<td>NA</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>Infarct surface area, cm²</td>
<td>NA</td>
<td>0.91±0.08</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>91±3.7</td>
<td>65±3.6†</td>
</tr>
<tr>
<td>LV dP/dt, mm Hg/s</td>
<td>2937±328</td>
<td>1560±218†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>10±2.4</td>
<td>19±2.7*</td>
</tr>
<tr>
<td>RV systolic pressure, mm Hg</td>
<td>27±1.4</td>
<td>38±1.7†</td>
</tr>
<tr>
<td>RV weight mg/g body wt</td>
<td>0.69±0.06</td>
<td>0.73±0.30</td>
</tr>
<tr>
<td>Tracer ET-1 extraction, %</td>
<td>63.3±1.5</td>
<td>40.9±4.5†</td>
</tr>
<tr>
<td>MV ET-1, pg/mL</td>
<td>1.77±0.22</td>
<td>2.77±0.56</td>
</tr>
<tr>
<td>Aortic ET-1, pg/mL</td>
<td>1.47±0.16‡</td>
<td>2.77±0.59*</td>
</tr>
</tbody>
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LV indicates left ventricle; LVEDP, LV end-diastolic pressure; and RV, right ventricle.

*P<0.05 and †P<0.01 vs sham.
‡P<0.05 vs MV ET-1 levels.
There was no significant difference between the mean gradients. In the isolated lungs perfused at a constant flow rate of 10 mL/min, the perfusion pressure was significantly higher in the infarct group (10.6 ± 0.8 mm Hg) than in the sham group (6.9 ± 0.51 mm Hg, \( P = 0.001 \)). Mean percent tracer ET-1 extraction was reduced by \( \approx 22\% \) (\( P < 0.001 \), Table). Figure 2 demonstrates that both aortic and MV ET-1 levels were strongly inversely correlated with pulmonary ET-1 extraction. The relationship is best described by a curvilinear relation and was fitted by a natural logarithmic function: \([\text{ET-1}] = 14.3 - 3.167 \times \ln(\text{Ext}) \) \( (r^2 = 0.83, P < 0.001) \), where Ext is pulmonary ET-1 extraction. There was no significant correlation between both aortic and MV ET-1 levels and left ventricular end-diastolic pressure, infarct weight or size, and right ventricular systolic pressure.

**Discussion**

We used the rat myocardial infarction model to evaluate the effect of HF on pulmonary ET-1 clearance. Rats with moderate infarct size and mild pulmonary hypertension demonstrated a reduction in the capacity to clear ET-1 from the circulation. The reduced ET-1 clearance was closely and inversely correlated with circulating ET-1 levels and was associated with the loss of ET-1 gradient across the pulmonary circulation that we observed in control rats. These data suggest that the lungs, by failure to clear ET-1 in MV blood, contribute to the increase in circulating ET-1 levels found in this model of HF.

**Pulmonary Metabolic Functions**

The pulmonary vascular bed with its large vascular surface area accommodating the whole cardiac output is recognized to be an important modulator of various circulating vasoactive amines and peptides. Congestive HF causes pulmonary venous congestion with initially passive pulmonary hypertension that may eventually become reactive and irreversible, depending on the severity and duration of HF. The effect of HF on the metabolic properties of the pulmonary vasculature, however, has never been evaluated. Modifications of these properties may not only contribute to the development of pulmonary hypertension itself but also could modulate the levels of circulating mediators known to adversely affect the evolution of HF.

The pulmonary vascular bed is the most important site for circulating ET-1 clearance in rats. It is also important in humans, with a mean first-pass ET-1 extraction of \( \approx 50\% \). This clearance is achieved through the endothelial ET\(_B\) receptor and as such can be considered a newly recognized endothelial cell function. The ET\(_A\) receptor effectively modulates extracellular ET-1 levels in cultured cells. In conscious rats, the ET\(_A\) antagonists BQ-123 and FR-139317 did not affect plasma ET-1 levels, whereas the nonspecific ET\(_B\)/ET\(_A\) blocker RO-462005 increased circulating ET-1 levels by 200%. In humans with HF, a single dose of the mixed ET\(_B\)/ET\(_A\) blocker bosentan caused a 2-fold increase in already elevated circulating ET-1 levels.

We found a reduced pulmonary ET-1 clearance in rats with HF, the first time that this novel pulmonary metabolic function has been evaluated in a pathological condition. Our findings suggest that the reduced pulmonary clearance of ET-1 is an important contributor to the elevated concentrations of plasma ET-1 found in HF. Other mechanisms, such as an increase in the pulmonary release of ET-1 or a contribution from the systemic circulation (liver, kidney, and other organs), cannot be excluded, and their relative importance compared with a reduced pulmonary clearance will require additional studies. However, we failed to find any significant correlation between ET-1 levels and infarct size, left ventricular end-diastolic pressure, or right ventricular systolic pressure. These findings differ from those of previous investigators who found that in patients with HF, plasma ET-1 levels correlated independently with the severity of pulmonary hypertension and pulmonary vascular resistance. The infarcted rats in our experiments developed only mild pulmonary hypertension with no right ventricular hypertrophy. This suggests that the reduced pulmonary clearance of ET-1 occurs before the development of more severe pulmonary hypertension and as such could be an early and sensitive marker of pulmonary vascular endothelial dysfunc-
tion associated with HF. The relationship between ET-1 concentrations and pulmonary ET-1 clearance is best described by a curvilinear relationship (Figure 2). In patients with HF and comparably more severe pulmonary hypertension, Cody et al. also found a curvilinear relationship between ET-1 concentrations and pulmonary artery pressure, supporting the primary role of the pulmonary vascular bed in the increase of circulating ET-1.

Pathogenesis and Significance of Reduced Pulmonary ET-1 Clearance

The mechanism of the reduced pulmonary ET-1 clearance remains speculative at this point. A possible explanation is that pulmonary congestion may result in a downregulation or desensitization of endothelial ETB. The reported reduction in ETB mRNA expression in the monocrotaline model of pulmonary hypertension and the recently reported reductions in ETB receptor density, as well as ETB mRNA expression and ETB receptor protein level by Western hybridization of whole-lung homogenates from infarct rats, support that hypothesis. Another possible explanation would be receptor occupancy by endogenous ET-1. Indeed, 125I-labeled ET-1 is avidly bound by the isolated rat pulmonary circulation and is not displaced by subsequent infusion of cold ET-1 at a concentration of 5×10−9 mol/L. The demonstration of an increased preproendothelin-1 mRNA expression and ET-1 immunoreactivity by immunohistochemistry in lungs from rats with congestive HF secondary to myocardial infarction is compatible with this hypothesis.

The reduced bioavailability of the pulmonary endothelial ETB may adversely affect both pulmonary and systemic vascular reactivity. The endothelial ETB receptor causes vasodilatation through the release of nitric oxide and prostacyclin. The ETB receptor has been shown to attenuate the vasodilatation through the release of nitric oxide and prostacyclin and the production of thromboxane A2. The ETB receptor may adversely affect both pulmonary and systemic circulation in severe chronic heart failure. The ETB receptor has been found to be downregulated in the lungs of patients with chronic heart failure. The ETB receptor may play a role in the development of pulmonary hypertension in patients with chronic heart failure. The ETB receptor has been found to be downregulated in the lungs of patients with chronic heart failure. The ETB receptor may play a role in the development of pulmonary hypertension in patients with chronic heart failure.

Conclusions

Congestive HF secondary to myocardial infarction causes a reduction in pulmonary clearance of ET-1. This reduced clearance contributes to the increase of circulating ET-1 levels. Additional studies are needed to determine whether this reduced clearance contributes not only to the development of pulmonary hypertension but also to deterioration of systemic hemodynamics.

Acknowledgments

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