Plasma Endothelin Correlates With the Extent of Pulmonary Hypertension in Patients With Chronic Congestive Heart Failure

Robert J. Cody, MD; Garrie J. Haas, MD; Philip F. Binkley, MD; Quinn Capers, MD; and Robert Kelley, MS

Background. Endothelin is a family of potent vasoconstrictor peptides of vascular endothelial origin. Although it has been proposed that the vasoconstrictor effects of endothelin are produced at the local vascular level, increased plasma concentration of endothelin has been identified in cardiovascular disorders.

Methods and Results. We tested whether immunoreactive endothelin-1 could be detected by radioimmunoassay in plasma of congestive heart failure patients and whether levels correlated with hemodynamic characteristics. Twenty congestive heart failure patients (New York Heart Association class II–IV) were sampled in the morning after an overnight fast, before medication. Cardiac index was decreased to 2.14±0.45 l/m², and pulmonary wedge pressure was increased to 22±7 mm Hg. The ranges of pulmonary pressures were: systolic, 22–100 mm Hg; mean, 13–61 mm Hg, and diastolic, 8–42 mm Hg. The endothelin-1 level was 9.07±4.13 pg/ml (range, 4–19 pg/ml), which was increased compared with 12 normals (3.7±0.6 pg/ml; range, 2.8–4.7 pg/ml); the difference was statistically significant (p<0.0001). Endothelin-1 significantly correlated with pulmonary pressures (systolic, r=0.78; mean, r=0.80; diastolic, r=0.77; all p<0.003) and pulmonary vascular resistance (r=0.65, p<0.01). Endothelin-1 strongly correlated with the resistance ratio (pulmonary vascular resistance/systemic vascular resistance) (r=0.88, p<0.0001). Stepwise multiple regression analysis confirmed the significance of these observations.

Conclusions. Elevated immunoreactive endothelin-1 specifically correlated with the extent of pulmonary hypertension in congestive heart failure patients. Whether endothelin-1 is a regional mediator of pulmonary hypertension or a marker for its occurrence requires additional evaluation. (Circulation 1992;85:504–509)

Endothelin is a vasoactive peptide with potent vasoconstrictor properties.1,2 In a recent study,3 endothelin was identified by radioimmunoassay in the plasma of patients with both primary and secondary pulmonary hypertension and was increased compared with normal subjects. Thus, the role of endothelin in cardiovascular disorders can be explored at the clinical level. Many patients with chronic congestive heart failure have increased pulmonary pressure and resistance4 and abnormal neurohormonal activity.5,6 However, individual neurohormonal factors have not demonstrated specific causality with the extent of pulmonary vasoconstriction. The current study was performed to determine whether endothelin could be detected in the plasma of patients with congestive heart failure and whether levels could be related to hemodynamic status. The findings of this study reveal that plasma endothelin specifically correlates with the extent of pulmonary hypertension and vasoconstriction in chronic congestive heart failure.

Methods

Hemodynamic Evaluation

The study consisted of 20 patients with mild-to-severe chronic congestive heart failure. All patients were undergoing investigative hemodynamic evaluation of their congestive heart failure, for which they had given written consent; these studies were approved by the Committee on Human Subjects and
monary vascular resistance/systemic vascular resistance) provides a means by which pulmonary vascular resistance can be indexed to the influences of the systemic resistance. After appropriate mathematical calculation, the resistance ratio represents the driving pressure across the pulmonary circulation divided by the driving pressure across the systemic circulation (PAP-PWP/MAP-RAP). We therefore used the resistance ratio in the current study. The rationale for this approach has been described previously.4

Endothelin Assay

Blood samples for plasma endothelin-1 were obtained at the time of hemodynamic determination. Four milliliters of arterial blood was collected into chilled tubes containing 4% EDTA and 2,000 KIU aprotinin. Samples were kept on ice and were then centrifuged at 4°C. All separated plasma samples were then immediately stored at −80°C until analysis. At the time of analysis, plasma samples were thawed, immediately acidified with an equal volume of 0.1% trifluoroacetic acid (TFA), and centrifuged at 2,000g at 4°C for 15 minutes to remove proteolytic activity. The supernatant was extracted using Sep-Column C18 cartridges (Peninsula Laboratories) that had been activated with 60% acetonitrile in 0.1% TFA, followed by washing with 0.1% TFA. After addition of samples to the cartridges, endothelin was eluted with 60% acetonitrile in 0.1% TFA. Using a vacuum centrifugal concentrator, samples were dried overnight and stored at −80°C. Recovery of endothelin-1 during extraction was 82% as assessed by calculating recovery of known amounts of endothelin-1, which had been added to plasma. Radioimmunoassay was performed on reconstituted samples by using rabbit anti-human endothelin-1 (Peninsula Laboratories). The antibody used in this study primarily recognizes endothelin-1, with 100% cross-reactivity. There is also 17% cross-reactivity with human big endothelin and 7% cross-reactivity with endothelin-2 and endothelin-3. There is no cross-reactivity with human atrial natriuretic peptide, brain natriuretic peptide, the angiotensins, vasoactive intestinal peptide, vasopressin, or ACTH. The results given in this study should therefore be considered immunoreactive endothelin-1, in consideration of the limited cross-reactivity. After overnight incubation at 4°C, 125I-endothelin-1 was added, and incubation continued an additional 20 hours at 4°C. Immune complexes were precipitated with goat anti-rabbit serum, and the precipitates were counted for radioactivity. Intra-assay coefficient of variation was 7%; the lower limit of detection was 0.5 pg/ml.

FIGURE 1. Plot shows comparison of plasma immunoreactive endothelin-1 in normal subjects and congestive heart failure patients (CHF). There was a threefold increase of mean endothelin-1 values in CHF subjects compared with normal subjects.
TABLE 1. Regression Data: Plasma Endothelin-1 (Ordinate) Versus Systemic and Pulmonary Hemodynamics (Abscissa)

<table>
<thead>
<tr>
<th>Endothelin-1 vs.</th>
<th>Regression</th>
<th>$r$</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate</td>
<td>Linear</td>
<td>0.21</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Arterial pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>Linear</td>
<td>0.03</td>
<td>0.0007</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic</td>
<td>Linear</td>
<td>0.05</td>
<td>0.0002</td>
<td>NS</td>
</tr>
<tr>
<td>Mean</td>
<td>Linear</td>
<td>0.05</td>
<td>0.0002</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>Polynomial</td>
<td>0.74</td>
<td>0.55</td>
<td>&lt;0.002</td>
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<tr>
<td>Diastolic</td>
<td>Polynomial</td>
<td>0.76</td>
<td>0.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>Polynomial</td>
<td>0.78</td>
<td>0.6</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Right atrial pressure</td>
<td>Linear</td>
<td>0.56</td>
<td>0.31</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pulmonary wedge pressure</td>
<td>Linear</td>
<td>0.4</td>
<td>0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac index</td>
<td>Linear</td>
<td>0.2</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Stroke volume index</td>
<td>Linear</td>
<td>0.3</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Systemic vascular resistance</td>
<td>Linear</td>
<td>0.17</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary vascular resistance</td>
<td>Polynomial</td>
<td>0.67</td>
<td>0.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Resistance ratio</td>
<td>Polynomial</td>
<td>0.88</td>
<td>0.77</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Statistical Methods

Comparison of normal and congestive heart failure endothelin levels was by two-tailed, unpaired $t$ test. To identify relevant relations, regression analysis of hemodynamic values with plasma endothelin was performed. Where appropriate, polynomial regression was also performed. To confirm the observations made by independent regression analysis, we performed forward stepwise regression analysis to select appropriate hemodynamic variables producing the highest partial correlation with plasma endothelin-1. All values are expressed as mean±1 SD.

Results

Plasma Endothelin

In normal subjects, plasma endothelin-1 was 3.7±0.6 pg/ml (range, 2.8–4.7 pg/ml). In congestive heart failure, plasma endothelin-1 was 9.1±4.1 pg/ml (range, 4.0–19 pg/ml), $p<0.0001$ compared with normals (Figure 1). There was no correlation of age and plasma endothelin-1 for normal subjects ($r=0.02$) or heart failure patients ($r=0.14$).

Hemodynamic Characteristics of Congestive Heart Failure

The following values represent the hemodynamic characteristics of the heart failure population; range of values for each parameter is given in parentheses. Heart rate was 83±1 beats per minute (54–109 beats per minute). Arterial pressures were: systolic, 125±20 mm Hg (95–174 mm Hg); diastolic, 71±8 mm Hg (58–89 mm Hg); and mean, 88±12 mm Hg (70–111 mm Hg). Pulmonary artery pressures were: systolic, 56±21 mm Hg (22–100 mm Hg); diastolic, 26±9 mm Hg (8–42 mm Hg); and mean, 36±13 mm Hg (13–61 mm Hg). Right atrial pressure was 10±6 mm Hg (3–22 mm Hg), and pulmonary wedge pressure was 22±7 mm Hg (7–34 mm Hg). Cardiac index was 2.14±0.45 l/min/m² (1.33–3.14 l/min/m²); stroke volume index was 26±6 ml/m² (18–39 ml/m²). Systemic vascular resistance was 1,638±491 dyne · sec/cm² (967–2,640 dyne · sec/cm²), and pulmonary vascular resistance was 285±178 dyne · sec/cm² (26–693 dyne · sec/cm²). The resistance ratio was 0.18±0.1 (0.02–0.38).

Hemodynamic Correlates of Plasma Endothelin in Congestive Heart Failure

Data derived from regression analysis are summarized in Table 1. There were significant correlations of endothelin-1 with pulmonary artery pressures, right atrial pressure, pulmonary vascular resistance, and resistance ratio. Right atrial pressure was significantly correlated ($r=0.56$, $p<0.01$) with an index of determination of 0.31. Although significant by either linear or polynomial regression analysis, pulmonary artery pressures (Figure 2) and resistance ratio (Figure 3) were best described by nonlinear polynomial regression. The index of determination ($r^2$) indicated that at least 55% of the variability of pulmonary artery pressures was related to the variability of plasma endothelin-1. Seventy-seven percent of the variability of the resistance ratio was related to the variability of plasma endothelin, with a correlation of 0.88 ($p<0.0001$). Overall, there were no significant correlations of endothelin-1 with other systemic hemodynamic variables.

To verify the predominant correlation of plasma endothelin-1 with pulmonary hemodynamic variables, we performed stepwise multiple regression analysis. First, all 14 variables given in Table 1 were considered. With this approach, only the resistance ratio was selected by the computation for the regression equation ($r=0.86$; $r^2=0.73$). However, systemic vascular resistance, pulmonary vascular resistance, and the resistance ratio are derived
variables, the first two of which are used for the derivation of the resistance ratio. Therefore, stepwise regression analysis was repeated with 11 variables, excluding the three resistance variables. The results of this analysis are given in Table 2. This computation chose mean pulmonary artery pressure, pulmonary wedge pressure, and mean systemic arterial pressure for the regression equation. The percent of explained variance (partial $r^2$) in plasma endothelin-1 was accounted for by the variance in mean pulmonary artery pressure (50%), pulmonary wedge pressure (19%), and mean systemic arterial pressure (8%). The overall equation, using these variables, produced a correlation of $r=0.88$ and $r^2=0.77$ with plasma endothelin-1. By this approach, variance in right atrial pressure did not enter into the regression equation.

**Discussion**

We have demonstrated that endothelin-1 can be measured in human plasma. In view of the limited cross-reactivity with other peptide components of the endothelin family, however, this should be considered immunoreactive endothelin-1. The normal values in our study are consistent with previously published values.\(^4,^8\) Proteolytic digestion of endothelin-1 is minimized by collection and centrifugation of samples at low temperature. Addition of aprotinin inhibits proteolytic and endopeptidase degradation, and acidification of samples after thawing, with subsequent centrifugation, removes proteolytic activity. The somewhat lower values for normal in some studies\(^9\) may be due to variations on this methodology. The current data indicate that plasma endothelin-1 is increased in patients with chronic congestive heart failure compared with normal subjects. The mean value of endothelin-1 in heart failure was threefold greater than normal subjects, and the range of values demonstrates minimal overlap between the normal population and the heart failure population. Although some congestive heart failure patients have relatively low plasma endothelin-1 values, this was related to both relatively low pulmonary pressure and relatively low pulmonary vasoconstriction. The range of pulmonary pressures (systolic, 22–100 mm Hg) exhibited by the congestive heart failure patients in this study is broad, as is likely to be encountered in the clinical congestive heart failure population. The difference in plasma endothelin-1 between heart failure and normals was not a function of age, as mean values for the two groups were similar and there was no correlation between age and endothelin values for either normal subjects or congestive heart failure patients or for the combined data set.

There was a strong and specific correlation of plasma endothelin-1 with pulmonary hemodynamic variables. Pulmonary systolic, mean, and diastolic pressures were all highly correlated with plasma endothelin-1, as was pulmonary vascular resistance. Because it is virtually impossible to isolate the pulmonary circulation in intact subjects, one can nonetheless index pulmonary resistance to simultaneous systemic vascular resistance by using the resistance ratio.\(^4,^7\) This places the pulmonary driving pressure within the framework of simultaneous systemic arterial driving pressure. The importance of this relation to plasma endothelin-1 in the current study was identified by both univariate regression and stepwise multiple regression analysis. In the latter analysis, variance of mean pulmonary artery pressure and pulmonary wedge pressure (the numerator variables
of the resistance ratio) accounted for a full 69% of the variance in plasma endothelin-1. Variance of mean systemic arterial pressure added an additional 8%. This suggests a specific relation between the pulmonary circulation and plasma endothelin-1 in the congestive heart failure population. This concept is supported by studies in pulmonary hypertension and basic experimental observations.

Observations by Stewart and coworkers\(^5\) in patients with primary and secondary pulmonary hypertension closely parallel the observations in the current study. They observed increased plasma endothelin-1 values in both patient groups, yet none of their patients with secondary pulmonary hypertension had chronic congestive heart failure as an etiology of increased pulmonary artery pressure. Thus, our observations are novel. In vitro and in vivo studies have demonstrated that endothelin is a potent constrictor of both pulmonary arteries and veins, with a greater effect on pulmonary arteries.\(^9\) Furthermore, administration of endothelin results in a time-dependent increase of pulmonary artery pressure.\(^10\)-\(^13\) Similar observations have also been made with human pulmonary arteries studied in vitro.\(^14\) As recently reviewed by Ryan,\(^15\) pulmonary endothelial cells are rich in receptors for vasoconstrictor substances such as endothelin; therefore, the endothelial lining of pulmonary vessels can mediate changes in vascular tone. Formal clearance studies of endothelin-1 have been performed.\(^16\) These data indicate that 82% of radiolabeled endothelin-1 injected into a jugular vein is cleared by the pulmonary circulation, indicating specific receptor activity. Clearance by other tissues was equal to or less than 10%. Furthermore, endothelin is actively produced by cultured bovine pulmonary endothelial cells.\(^17\) Thus, although endothelin uptake from plasma and production by pulmonary endothelial cells is supported by previous reports, additional studies are required to characterize the relation of plasma endothelin-1 to the pulmonary circulation. In human studies, for example, simultaneous plasma sample acquisition from multiple vascular sites may provide further insight into production or clearance of endothelin by specific vascular beds. Stewart and colleagues\(^5\) observed only small differences between peripheral arterial and venous samples. In the current study, blood samples for endothelin in normal subjects were drawn from a peripheral vein, whereas a peripheral arterial sample site was used for the congestive heart failure patients. This is a potential limitation; however, this does not have an adverse influence on the strong correlation of plasma endothelin with pulmonary hemodynamics in the heart failure population. Nonetheless, detailed studies evaluating clearance or production of endothelin across the pulmonary and systemic vascular beds must be performed. Additionally, we cannot determine from the current study whether the increase of plasma endothelin-1 represents a biological marker for the occurrence of pulmonary hypertension in congestive heart failure or whether endothelin-1 contributes to the pathophysiology of the disorder as a specific mechanism of vasoconstriction. Specific pharmacological inhibitors of endothelin or biochemical analogues will be necessary to address this issue. Abnormal neurohormonal activity is a contributor to the pathophysiology of congestive heart failure.\(^5\),\(^6\) Each of these separate pathways, such as the renin-angiotensin system, catecholamines, vasopressin, and atrial natriuretic factor, may influence regional and overall cardiovascular function in heart failure. However, a direct effect of these traditional neurohormonal pathways on the pulmonary hypertension and pulmonary vasoconstriction of congestive heart failure has never been established. Thus, the close correlation of plasma endothelin with the pulmonary circulatory abnormalities of congestive heart failure is unique. Pulmonary hypertension in patients with congestive heart failure remains a major concern. Relative increases of pulmonary pressure and resistance can limit the effectiveness of vasodilator therapy\(^18\) and can exclude patients from candidacy for cardiac transplantation. Therefore, additional specific studies assessing

**Table 2. Stepwise Multiple Regression Analysis of Hemodynamic Variables That May Influence Plasma Endothelin**

<table>
<thead>
<tr>
<th>Variable</th>
<th>β-Coefficient±SEM</th>
<th>F</th>
<th>Partial (r^2)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pulmonary artery pressure</td>
<td>0.55±0.08 (mm Hg)</td>
<td>43.08</td>
<td>0.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pulmonary wedge pressure</td>
<td>-0.6±0.14 (mm Hg)</td>
<td>17.47</td>
<td>0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean systemic arterial pressure</td>
<td>-0.11±0.05 (mm Hg)</td>
<td>5.77</td>
<td>0.08</td>
<td>&lt;0.04</td>
</tr>
</tbody>
</table>

Overall regression equation: Intercept coefficient = 12.47, \(r=0.88\), \(r^2=0.77\), \(F\) test = 18.06.
the relation of plasma endothelin-1 with the pulmonary circulation are warranted.

References


Key Words • endothelin • pulmonary hypertension • congestive heart failure
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