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Prognostic value of plasma endothelin-1 in patients with chronic heart failure

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Aims Endothelin-1 is a potent vasoconstrictive and multifunctional peptide. Elevated concentrations have been reported in congestive heart failure. We hypothesized that the level of endothelin-1 in plasma is a prognostic marker in congestive heart failure.

Methods and results Plasma levels of endothelin-1 were measured by radioimmunoassay in 120 congestive heart failure patients with ischaemic or non-ischaemic cardiomyopathy (mean ejection fraction 28 ± 11%, in New York Heart Association (NYHA) functional class I: 21, class II: 35, class III: 61, class IV: 3). During a median follow-up of 361 ± 338 days, 14 cardiac deaths occurred. In the univariate Cox model, endothelin-1 was the most powerful prognostic marker among the variables tested ($P=0.0001$). A multivariate model, including plasma atrial natriuretic peptide and noradrenaline, NYHA class, age, and echocardiographic left ventricular end-diastolic diameter index was highly predictive of mortality ($P=0.00008$), but only endothelin-1 remained significantly associated with outcome ($P=0.02$). Patients with plasma endothelin-1 ≥ 5 pg . ml^{-1} had a higher mortality rate than those with endothelin-1 <5 pg . ml^{-1} (21% vs 4%, $P=0.001$).

Conclusion Our results suggest that elevated endothelin-1 plasma levels are associated with a poor prognosis and routine plasma endothelin-1 determination provides important prognostic information in mild to moderate heart failure.

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Key Words: Endothelin-1, chronic heart failure, prognosis.

Introduction

There is considerable clinical and experimental evidence that neurohormonal activation is deleterious and may contribute to the progression of left ventricular dysfunction in heart failure. Increased levels of plasma norepinephrine and atrial natriuretic peptide are associated with a poor prognosis in heart failure[1,2]. Endothelin-1 is a potent endothelial cell-derived peptide with multifunctional properties including venous and arterial vasoconstrictor properties, the ability to modulate inotropy, and to induce hypertrophy and gene expression[3–5]. Elevated plasma levels of endothelin-1 have been reported in heart failure[6,7]. Since endothelin-1 may contribute to haemodynamic deterioration by a potent vasoconstrictive action, we hypothesized that persistent high levels in chronic congestive heart failure may be associated with a poor prognosis.

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Methods

Patients

One hundred and twenty consecutive patients referred to our department because of a prior history of congestive heart failure due to systolic dysfunction were included in the study on condition that echocardiographic left ventricular end-diastolic diameter index was ≥ 34 mm . m^{-2} and that left ventricular ejection fraction was $\leq 50\%$. Ejection fraction was assessed by radionuclide ventriculography, contrast ventriculography or two-dimensional echocardiography. All patients were in a clinically stable condition and on constant therapy. Patients were excluded if they had had an acute myocardial infarction within 3 months, had valvular disease requiring surgery, planned cardiac surgery, significant liver or renal disease, or other life threatening disorders. All participants gave informed consent. Patients underwent clinical examination, echocardiography and the following plasma neurohormones were measured: endothelin-1, noradrenaline and atrial natriuretic peptide. Exercise maximal oxygen consumption measurement was available in only 96 patients, as 24

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patients were unable to practise a symptomatic maximal exercise test which was limited by heart failure-related symptoms.

After entering the study, patients were followed-up. The date of death was registered and the duration of follow-up calculated. Patients who underwent transplantation were censored at the time of the transplantation.

Blood sampling procedures and hormonal assays

Blood samples, drawn by direct venipuncture early in the morning before medication and after at least 30 min of supine rest, were immediately placed on ice and centrifuged at 4 °C. Plasma samples were stored at − 20 °C until assay. Plasma endothelin-1 concentrations were determined by use of a radioimmunoassay method performed in our laboratory as previously described[8]. Cross-reactivity with propeptide big-endothelin was 0·46. No significant cross-reactivity to other unrelated peptides (i.e. atrial natriuretic peptide, vasopressin, angiotensin 2) was reported. Method sensitivity was 0·25 pg . tube^{-1}, the intra-assay coefficient variation was 6·1%, and the inter-assay coefficient of variation was 15%. Our reference values were 3·5 ± 0·9 pg . ml^{-1} (n=20). Plasma catecholamines were measured by a radioenzymatic method using the enzyme catechol-o-methyl transferase; our reference values were 250 ± 50 pg . ml^{-1} (n=20). Plasma atrial natriuretic peptide (C-terminal fragment 99–126) concentrations were measured by radioimmunoassay; our reference values were 12·4 ± 4 pg . ml^{-1} (n=20).

Statistical analysis

Data are expressed as mean values ± SD. Survival was analysed with the Cox proportional-hazards model. Patients who underwent transplantation were censored at the time of the transplantation. Variables identified as significantly associated with outcome were then included in a multivariate analysis. Kaplan–Meier estimates of the survival functions were plotted for high and low plasma endothelin-1 levels with the number rounded up to the median as a cut-off point. Differences between survival curves were tested for significance by the log-Rank method. Statistical comparisons between New York Heart Association (NYHA) functional class groups were performed using analysis of variance followed by Neuman Keuls' test. Differences between survivors and deaths were also tested by using the Student's t-test. Linear regression analysis was used to assess the relationship between continuous variables. Significance was set at a P value <0·05.

Results

Table 1 summarizes patients' baseline clinical and haemodynamic characteristics. Although therapy was not standardized, most patients were maintained on a regimen including furosemide and angiotensin-converting enzyme inhibitors. Table 2 shows the baseline hormonal level of endothelin-1, atrial natriuretic peptide and noradrenaline.

Table 1 Baseline clinical and haemodynamic data

Characteristics	
Sex: Male/female	100/20
Age (years)	52 ± 11
New York Heart Association functional class	
Class I/II	21/35
Class III/IV	61/3
Aetiology of heart failure	
Idiopathic dilated cardiomyopathy	92 (77%)
Chronic coronary artery disease	23 (19%)
Anthracycline-induced cardiomyopathy	2 (1·7%)
Aortic regurgitation	3 (2·5%)
Left ventricular echocardiographic end-diastolic diameter index (mm . m^{-2})	39 ± 6
Left ventricular ejection fraction (%)	28 ± 11
Treatment	
Diuretics	99 (83%)
Angiotensin-converting enzyme inhibitors	105 (88%)
Vasodilators	53 (44%)
Digitalis	52 (43%)
Amiodarone	40 (33%)
Systolic blood pressure (mmHg)	124 ± 21
Heart rate (beats . min^{-1})	85 ± 18
Peak exercise oxygen consumption (ml . min^{-1} kg^{-1}), n=96)	15·2 ± 6·1

Values are as mean ± SD.

Table 2 Baseline hormonal data

Hormone levels	Values
Noradrenaline (pg . ml^{-1})	550 ± 242
Atrial natriuretic peptide (pg . ml^{-1})	169 ± 139
Endothelin-1 (pg . ml^{-1})	4·7 ± 1·6

Values are as mean ± SD.

During a median follow-up of 361 ± 338 days (15 days to 3·8 years), 14 cardiac deaths occurred (three sudden deaths and 11 end-stage heart failure deaths) and eight patients underwent transplantation. No patient was lost to follow-up. Univariate Cox proportional hazards regression analysis showed that endothelin-1 was the most powerful marker among the variables tested (Table 3).

A multivariate model including the six variables identified as significant predictors of outcome in the previous analysis (except maximal oxygen consumption), was highly predictive of mortality (P=0·00008), but only the endothelin-1 plasma level remained significantly associated with outcome (P<0·02). Estimated survival curves for groups with various plasma endothelin-1 values are shown in Fig. 1. Patients (n=51) with an endothelin-1 plasma level greater than 5 pg . ml^{-1} had a significantly higher mortality rate

Table 3 Univariate relation between various clinical and biochemical variables and mortality in heart failure patients according to a Cox proportional-hazard model

Variables	P
Endothelin-1	0·0001
NYHA	0·0007
Atrial natriuretic peptide	0·006
LV echocardiographic end-diastolic diameter index	0·007
Noradrenaline	0·01
Age	0·02
Maximal oxygen consumption (n=96)	0·03
Ejection fraction	0·13

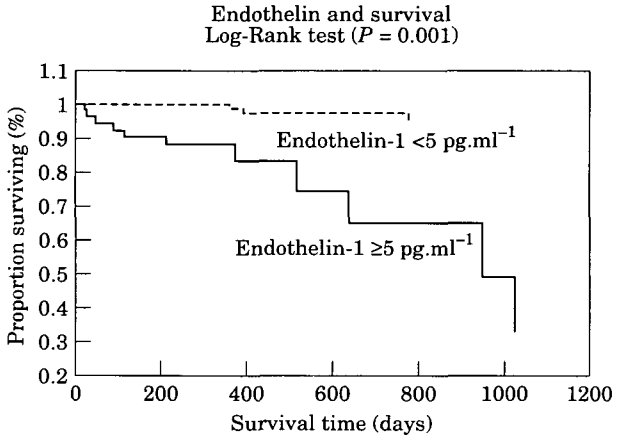

Figure 1 **Kaplan–Meier survival plot for patients with chronic heart failure subdivided into two groups according to the number rounded up to the median of plasma endothelin-1 (5 pg . ml^{-1}).**

than did those (n=69) with a lower circulating level (22% vs 4%, P=0·001, log-rank test). Patients who died during the follow-up had higher plasma endothelin-1 levels (6·05 ± 1·67 pg . ml^{-1} vs 4 53 ± 1·57 pg . ml^{-1}, P=0·001) and atrial natriuretic peptide levels (239 ± 106 pg . ml^{-1} vs 159 ± 141 pg . ml^{-1}, P=0·04) than living patients. Noradrenaline levels tend to be lower in surviving patients but the difference was not statistically significant (537 ± 292 pg . ml^{-1} vs 647 ± 233 pg . ml^{-1}, P=0·11). Endothelin-1 increased with severity of heart failure according to NYHA functional class (ANOVA, P=0·0004). Patients in NYHA functional class II (4·2 ± 1·6 pg . ml^{-1}) had significantly lower plasma endothelin-1 levels than patients in class III (5·1 ± 1·6 pg . ml^{-1}, P=0·008) and in class IV (7·1 ± 2·5 pg . ml^{-1}, P=0·006), but similar levels to patients in class I (3·9 ± 0·8 pg . ml^{-1}, P=0·57).

Discussion

The high mortality rate in heart failure and the difficulties in selecting patients for heart transplantation have increased the need for prognostic markers. Several indices of prognosis have been identified in heart failure: the New York Heart Association functional class, haemodynamic parameters, and functional capacity assessed by the determination of peak oxygen consumption on exercise[9,12]. Moreover, the degree of neurohormonal stimulation has been reported as a strong independent predictor of mortality in heart failure patients: elevated plasma levels of noradrenaline, of 99-126 ANP and more recently of the N-terminal fragment pro-ANP are associated with a poor prognosis in heart failure and in post myocardial infarction[1,2,9,13]. More recently, the endothelium-derived 21 amino acid peptide endothelin has been associated with mortality after myocardial infarction[14]. Similarly, high plasma levels of both endothelin-1 and of the propeptide big-endothelin have been reported as strong predictors of mortality in severe heart failure.[15,16] In our study we included patients with less severe conditions than in the two previous studies, including patients with asymptomatic left ventricular dysfunction; only three patients were in NYHA functional class IV. Our results suggest therefore that endothelin-1 is also an independent predictor of mortality in mild to moderate heart failure. Thus, elevated plasma levels of endothelin-1 and of the propeptide big-endothelin are markers of severity in various cardiovascular disorders including post myocardial infarction, pulmonary hypertension and chronic heart failure. In this last condition they provide additional independent prognostic information to that given by haemodynamic, functional or other hormonal parameters[15–17].

What is the potential pathophysiological significance of an increased endothelin-1 plasma level? The production of endothelin-1 in the heart is increased and the density of myocardial endothelin receptors is up-regulated in experimental heart failure[18]. Furthermore, the infusion of a selective endothelin-1 receptor antagonist reduces significantly contractility in this model, suggesting that endogenous endothelin-1 is involved in the maintenance of cardiac function in cardiac failure. Although short-term inotropic support by up-regulation of the endothelin pathway may be beneficial, prolonged stimulation of the endothelin system may induce important harmful modifications in the function or the structure of the cardiovascular system by either a local or systemic mechanism. Several factors may therefore contribute to the relationship observed between high plasma endothelin concentrations and mortality; (1) the potent vasoconstricting effects of endothelin-1 may further impair cardiac function (2) in vitro endothelin-1 stimulates contractility and may increase myocardial oxygen demand (3) endothelin-1 may induce remodelling of the heart; in vitro endothelin-1 stimulates hypertrophy and the expression of fetal genes in cardiac myocytes; moreover, endothelin-1 modulates collagen turnover in cardiac fibroblasts (4) endothelin-1 stimulates the release of catecholamines and other neurohormones (5) endothelin-1 secretion in the lung may regulate pulmonary vascular resistance in patients with chronic heart failure (6) increased endothelin-1 may reflect generalized

endothelial dysfunction in heart failure[4,5,7,19–22]. Finally, endothelin contributes to basal vascular tone in vivo as demonstrated in healthy subjects[23,24] and there is some evidence that this mechanism also operates in chronic heart failure: Kiowski *et al.* showed that a non-specific endothelin receptor antagonist, bosentan, reduced mean arterial pressure and systemic vascular resistance in congestive heart failure patients[25]. However, since there was no control group in this study we do not know to what extent bosentan acts against vasoconstriction in heart failure.

Interestingly, endothelin-1 plasma levels increased significantly from NYHA class I to class IV. This finding indicates that the level of endothelin-1 is not only a prognostic marker but also reflects the severity of the disease. It has been suggested that the elevation of plasma endothelin-1 in heart failure consists mainly of the pro-peptide big-endothelin[6]. The very low cross-reactivity of our assay excludes a significant presence of big-endothelin to explain our results. Our study was not designed to evaluate the influence of treatments on prognosis or neurohormonal stimulation and daily medications were not modified. Most patients received angiotensin-converting enzyme inhibitors. It is noteworthy that we observed increased endothelin-1 plasma levels in our patients, whereas in vitro experimental studies have suggested that the release of endothelin-1 is stimulated by angiotensin II concentrations[26]. This finding further suggests that endothelin-1 plasma levels reflect the severity of heart failure.

Study limitations

We observed only 14 deaths over a median follow-up period of 1 year. Although this finding limits the power of our results, it is certainly related to the fact that our population consisted mainly of patients with left ventricular asymptomatic dysfunction and mild to moderate heart failure. This subgroup of patients is at a lower risk of mortality than those with advanced heart failure. Another limitation is the fact that the normal range of plasma endothelin varies widely among laboratories[7,27]. This variation may reflect differences in sampling site as well as variations in the specificity of the endothelin antibodies employed for the radioimmunoassay. Therefore, the cut-off value used in the present study pertains only to our population and to our methodology of analysis. Use of the cut-off value of 5 pg . ml^{-1} for the assessment of the prognosis cannot be applied to a general population and to different laboratories without restriction.

Further studies are necessary to evaluate the influence of specific treatments on plasma endothelin-1. In particular, the fact that elevated plasma levels are associated with a poor prognosis emphasizes the need for the evaluation of various inhibitors of the endothelin-1 pathway in patients with congestive heart failure. Whether this modulation is effective in preventing progression of the disease or mortality will provide important information on the pathophysiological role of the endothelin system in congestive heart failure.

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References

[1] Cohn J, Levine T, Olivari MT *et al.* Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med 1984; 311: 819–23.
[2] Gottlieb S, Kukin ML, Ahern D, Packer M. Prognostic importance of atrial natriuretic peptide in patients with chronic heart failure. J Am Coll Cardiol 1989; 13: 1534–9.
[3] Yanagisawa M, Kurihara H, Kimura S *et al.* A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1998; 332: 411–15.
[4] Moraves CS, Reynolds EE, Stewart RW, Bond M. Endothelin is a positive inotropic agent in human and rat heart in vitro. Biochem Biophys Res Commun 1989; 159: 14–8.
[5] Ito H, Hirata Y, Hiroe M *et al.* Endothelin-1 induces hypertrophy with enhanced expression of muscle-specific genes in cultured neonatial rat cardiomyocytes. Circ Res 1991; 69: 209–15.
[6] Wei C, Lermann A, Rodeheffer R *et al.* Endothelin in human congestive heart failure. Circulation 1994; 89. 1580–6.
[7] Cody R, Haas G, Binkley P, Capers Q, Kelley R. Plasma endothelin correalates with the extent of pulmonary hypertension in patients with chronic congestive heart failure. Circulation 1992; 85: 504–9.
[8] Cacoub P, Dorent R, Nataf P *et al.* Plasma endothelin and pulmonary pressures in patients with congestive heart failure. Am Heart J 1993; 126: 1484–8.
[9] Cohn J, Johnson G, Shabetai R *et al.* Ejection fraction, peak exercise oxygen consumption, cardiothoracic ratio, ventricular arrhythmias, and plasma norepinephrine as determinants of prognosis in heart failure. Circulation 1993, 87 (Suppl VI): VI 5–VI 16.
[10] Francis G, Cohn J, Johnson G *et al.* Plasma norepinephrine, plasma renin activity, and congestive heart failure. Relations to survival and the effects of therapy in V-Heft II. Circulation 1993; 87 (Suppl VI): VI 40–VI 48.
[11] Fuster V, Gersh B, Giulliani E, Tajik A, Bradenburg R. The natural history of idiopathic dilated cardiomyopathy. Am J Cardiol 1981; 47: 525–31.
[12] Franciosa J, Wilen M, Ziesche S, Cohn J. Survival in men with severe chronic left ventricular failure due to either coronary artery disease or idiopathic dilated cardiomyopathy. Am J Cardiol 1983; 51: 831–6.
[13] Hall C, Rouleau J, Moyè L *et al.* N-terminal proartrial natriuretic factor. An independnt predictor of long-term prognosis after myocardial infarction. Circulation 1994; 89: 1934–42.
[14] Omland T, Lie RT, Aakvaag A, Aarsland T, Dickstein K. Plasma endothelin determination as a prognostic indicator of 1-year mortality after acute myocardial infarction. Circulation 1994; 89: 1573–9.
[15] Pacher R, Stanek B, Hülsmann M *et al.* Prognostic impact of big endothelin-1 plasma concentrations compared with invasive hemodynamic evaluation in severe heart failure. J Am Coll Cardiol 1996; 27: 633–41.
[16] Tsutamoto T, Hisanaga T, Fukai D *et al.* Prognostic value of plasma soluble intercellular adhesion molecule-1 and endothelin-1 concentration in patients with chronic congestive heart failure. Am J Cardiol 1995; 76: 803–8.
[17] Nootens M, Kaufmann E, Rector T *et al.* Neurohormonal activation in patients with right ventricular failure from pulmonary hypertension: relation to hemodynamic variable and endothelin levels. J Am Coll Cardiol 1995; 26: 1581–5.
[18] Sakai S, Miyauchi T, Sakurai T *et al.* Endogenous endothelin-1 participates in the maintenance of cardiac

function in rats with congestive heart failure. Marked increase in Endothelin-1 production in the failing heart. Circulation 1996; 93: 1214–22.

[19] Tsutamoto T, Wada A, Maeda Y, Adachi T, Kinoshita M. Relation between endothelin-1 spillover in the lungs and pulmonary vascular resistance in patients with chronic heart failure. J Am Coll Cardiol 1994; 23: 1427–33.

[20] Paulus W. Endothelial control of vascular and myocardial function in heart failure. Cardiovasc Drugs Ther 1994; 8: 437–46.

[21] Shubetai HE, McDonough PM, Harris AN *et al.* Endothelin induction of inositol phospholipid hydrolysis, sarcomere assembly and cardiac gene expression in ventricular myocytes: a paracrine mechanism for myocardial cell hypertrophy. J Biol Chem 1990; 65: 2130–2203.

[22] Guarda E, Katwa LC, Myers PR, Tyagi SC, Weber KT. Effects of endothelins on collagen turnover in cardiac fibroblasts. Cardiovasc Res 1993; 27: 2130–4.

[23] Haynes W, Strachan F, Webb D. Endothelin ET_A and ET_B receptors cause vasoconstriction of human resistance and capacitance vessels in vivo Circulation 1995; 92: 357–63.

[24] Haynes WG, Webb D. Contribution of endogenous generation of endothelin-1 to basal vascular tone. Lancet 1994; 344: 852–4.

[25] Kiowski W, Sutch G, Kim P *et al.* Evidence for endothelin-1 mediated vasoconstriction in severe chronic heart failure. Lancet 1995; 346: 732–6.

[26] Emori T, Hirata Y, Schichiri M, Marumo F. Secretory mechanism of immunoreactive endothelin in cultured bovine endothelial cells. Biochem Biophys Res Commun 1989; 160: 93–100.

[27] Stewart DJ, Cernacek P, Costello CB, Roulleau JL. Elevated endothelin-1 in heart failure and loss of normal response to postural change. Circulation 1992; 85: 510–17.