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Plasma adrenomedullin, a new independent predictor of prognosis in patients with chronic heart failure

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Background Adrenomedullin, a potent endogenous vasodilating and natriuretic peptide, may play an important role in the pathophysiology of chronic heart failure. Plasma levels of immunoreactive adrenomedullin were examined for prediction of prognosis in chronic heart failure.

Methods and Results Plasma levels of immunoreactive-ADM (ir-ADM) were measured by radioimmunoassay in 117 chronic heart failure patients with idiopathic or ischaemic cardiomyopathy (mean ejection fraction: $28 \pm 10\%$, in the NYHA functional class I/II/III/IV:8/73/29/7, and treated with ACE inhibitors and diuretics. Plasma levels of immunoreactive adrenomedullin were significantly increased in chronic heart failure patients by comparison to controls (618 ± 293 pg . ml^{-1} vs 480 ± 135 pg . ml^{-1}, $P=0{\cdot}01$). During the follow-up period (237 ± 137 days) 14 cardiovascular deaths and four urgent cardiac transplantations occurred. In the univariate Cox model, immunoreactive adrenomedullin plasma levels were related to prognosis ($P=0{\cdot}004$). A multivariate analysis including heart rate, systolic blood pressure, NYHA class, left ventricular ejection fraction, left ventricular echocardiographic end-diastolic diameter, plasma levels of immunoreactive adrenomedullin, endothelin-1, norepinephrine and atrial natriuretic peptide was performed: plasma levels of immunoreactive adrenomedullin ($P=0{\cdot}03$), of endothelin-1 ($P=0{\cdot}0001$), and systolic blood pressure ($P=0{\cdot}003$) were significantly associated with outcome.

Conclusion Our results suggest that elevated plasma levels of immunoreactive adrenomedullin are an independent predictor of prognosis in predominantly mild to moderate chronic heart failure treated by conventional therapy and provide additional prognostic information.
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Key Words: Adrenomedullin, chronic heart failure, prognosis.

Introduction

Human adrenomedullin, discovered in 1993, is a 52-amino-acid peptide with a structural homology related to calcitonin gene-related peptide and amilyn. Originally isolated from human pheochromocytoma cells, immunoreactive adrenomedullin has subsequently been detected in a wide variety of tissues including the adrenal medulla, brain, lung, kidney, gastrointestinal organs and heart[12]. Adrenomedullin is a potent endogenous natriuretic and vasodilating peptide and increases cardiac output[3]. These findings suggest that adrenomedullin may play an important role in the regulation of local and systemic vascular tone. This peptide could function as an atrial natriuretic peptide in the control of cardiorenal homeostasis and may influence cardiovascular function.

Congestive heart failure is a pathological state characterized by the activation of various neuroendocrine systems, with activation of vasodilating factors as opposed to coactivation of local and circulating vasoconstrictive and sodium-retaining factors. Increased levels of plasma adrenomedullin have been reported in heart failure[4]. Therefore adrenomedullin may play an important role in the pathophysiology of heart failure and in the progression of left ventricular dysfunction. However, the potential value of plasma adrenomedullin levels, as an indicator of cardiovascular prognosis in heart failure, is unknown. In this report adrenomedullin was examined for the prediction of prognosis in patients with chronic heart failure due to systolic dysfunction and compared with previously reported markers.

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Methods

Entry criteria

One hundred and seventeen patients with chronic heart failure and with a left ventricular ejection fraction of 45% or less were included in the study (from February 1997 to February 1998). The ejection fraction was assessed by radionucleide ventriculography or two-dimensional echocardiography. All patients were in a clinically stable condition and had been on constant therapy for at least 1 month. Patients were excluded if they had had an acute myocardial infarction within the previous 3 months, valvular disease requiring surgery, planned cardiac surgery, significant liver or renal disease or other life-threatening disorders. All participants in the study gave informed consent.

Baseline data collection

At the time of recruitment into the study, baseline clinical data were prospectively obtained from all patients. Systolic blood pressure and heart rate were measured in the supine position. A venous blood sample was taken for assessment of electrolyte concentration and renal and liver function. ECG and a chest roentgenogram were performed for all patients. Two-dimensional and M-mode echocardiography was performed using a standardized protocol in accordance with the American Society of Echocardiography Recommendations. Left ventricular cavity dimensions at end diastole were measured (M-mode) and left ventricular ejection fraction was obtained by 2D echocardiography.

Blood sampling procedures and hormonal assays

Blood samples were obtained in the morning after a supine rest of 30 min. They were drawn from an indwelling catheter into chilled glass tubes and immediately placed on ice. The plasma was separated by centrifugation at 2500 rpm at 4 °C and stored at −20 °C until analysed. Plasma immunoreactive adrenomedullin was measured by a direct radioimmunoassay method developed in our laboratory. The standard (100 µl) adrenomedullin of plasma was incubated in a sodium phosphate PH 7·4 buffer with 100 µl antiserum (Peninsula Laboratories RAS 9500 rabbit anti-human adrenomedullin 1-52). The mixture was incubated at 4 °C for 24 h. 2000 cpm . 50 μl^{-1} of ^{125}I-h adrenomedullin 1-52 (Amersham) were added to each reaction mixture and incubated for 24 h at 4 °C. To perform a second antibody separation, 100 µl of dilute goat anti-rabbit Ig G serum (GARGG-500) and 100 ml of dilute normal rabbit serum (NRS-500, Peninsula laboratories) were added. After 2 h at 20 °C, 500 µl of the radioimmunoassay buffer were then added. After centrifugation, radioactivity of the pellet was measured with a gamma counter (Packard). After serial dilution of plasma samples, the curves produced were parallel with the standard curve for h-adrenomedullin 1-52, indicating immunological identity of the standard and the samples. Cross-reactivity of various peptides was as follows: h-adrenomedullin 1-52, 100%; h-adrenomedullin 1-51, 0·5%; calcitonin gene-related peptide, 0%; endothelin-1, 0%; brain natriuretic peptide-32, 0%; atrial natriuretic peptide, 0%. Recovery of synthetic h-adrenomedullin 1-52 was 84·5%. The assay detection limit was 2·5 pg/tube. Normal values for plasma immunoreactive adrenomedullin were $48 \pm 13{\cdot}5$ pg/tube (n=31, mean age : 44 ± 10 years).

As described previously plasma endothelin-1 concentrations were measured by a radioimmunoassay method, with reference values of $3{\cdot}5 \pm 0{\cdot}9$ pg . ml^{-1}. Plasma norepinephrine was measured with a radioenzymatic method, with reference values of 250 ± 50 pg . ml^{-1}. Plasma atrial natriuretic peptide (C-terminal fragment 99-126) concentrations were measured by radioimmunoassay method, with reference values of $12{\cdot}4 \pm 4$ pg . ml^{-1}[5].

Statistical analysis

After entering the study, patients were followed up until 30 June 1998. The date of death or urgent transplantation was registered and the duration of follow-up calculated. Statistical analysis was performed using two steps: first, a univariate analysis was performed in order to study the relationships between survival time and potential risk factors. The variables studied were: age, sex, NYHA functional class, systolic blood pressure, heart rate, serum creatinine, plasma norepinephrine, plasma atrial natriuretic peptide, plasma endothelin-1, plasma adrenomedullin, echocardiographic left ventricular ejection fraction and end-diastolic diameter. These relationships were tested using the univariate Cox model for continuous variables and the log rank test for qualitative ones. In a second step, a multivariate regression analysis was performed using the Cox model. Variables significantly related to the outcome with a P value <0·10 were included in a stepwise regression analysis. Variables and risk ratios were standardized.

Statistical comparison of immunoreactive adrenomedullin between NYHA functional class groups were performed using analysis of variance followed by the Neuman Keuls Test. Linear regression analysis was used to assess the relationship between continuous variables Significance was set at a P value <0·05. Data are expressed as mean values ± SD.

Results

Population

We studied 117 ambulatory patients, 93 men and 24 women (55 ± 12 years, 21 to 79 years) with chronic

Table 1 Baseline clinical and haemodynamic data

Characteristics	
New York Heart Association functional class I/II/III/IV	8/73/29/7
Aetiology of heart failure	
Idiopathic dilated cardiomyopathy	75
Chronic coronary artery disease	33
Anthracycline-induced cardiomyopathy	2
Hypertension	4
Valvular disease	3
Treatment	
Diuretics	96
Angiotensin-converting enzyme inhibitors	106
Vasodilators	47
Digitalis	50
Betablockers	20
Amiodarone	36
Left ventricular ejection fraction (%)	28 ± 10
Left ventricular echocardiographic end-diastolic diameter (mm)	68 ± 6
Systolic blood pressure (mmHg)	123 ± 23
Heart rate (beats . min^{-1})	80 ± 15
Serum creatinine (pg . l^{-1})	12·0 ± 3·7
ir-Adrenomedullin (pg . ml^{-1})	618 ± 293
Norepinephrine (pg . ml^{-1})	570 ± 334
Atrial Natriuretic Peptide (pg . ml^{-1})	267 ± 225
Endothelin-1 (pg . ml^{-1})	4·7 ± 1·3

Values are as mean ± SD.

heart failure. Clinical data and neurohumoral results are given in Table 1. All patients were on treatment and most were maintained on a regimen including furosemide and an ACE inhibitor. Plasma immunoreactive adrenomedullin levels were higher in chronic heart failure patients than in control subjects (618 ± 293 pg . ml^{-1} vs 480 ± 135 pg . ml^{-1}, $P=0·01$). Patients had neurohormonal activation assessed by increased plasma levels of the following neurohormones (median, min–max): norepinephrine (515 pg . ml^{-1}, 130–2446 pg . ml^{-1}), endothelin-1 (4·5 pg . ml^{-1}, 1·1–8·5 pg . ml^{-1}), atrial natriuretic peptide (206 pg . ml^{-1}, 25–1290 pg . ml^{-1}), adrenomedullin (570 pg . ml^{-1}, 242–2080 pg . ml^{-1}).

Immunoreactive adrenomedullin plasma levels were similar in men and women (P=ns), did not correlate with age (P=ns), and were not influenced by the aetiology of heart failure (ischaemic vs idiopathic aetiology, P=ns). Immunoreactive adrenomedullin plasma level tended to be higher in severely affected patients (Class I and II: 608 ± 253 pg . ml^{-1} vs Class III and IV: 640 ± 390 pg . ml^{-1}; P=ns). There was no correlation between plasma concentrations of immunoreactive adrenomedullin and left ventricular ejection fraction ($r=0·05$, P=ns), or the left ventricular echocardiographic end-diastolic diameter ($r=0·03$, P=ns). There was no significant correlation between plasma concentrations of immunoreactive adrenomedullin and other circulating neurohormones (atrial natriuretic peptide, norepinephrine or plasma endothelin-1 plasma levels, P=ns).

Follow-up

During the follow-up period (237 ± 137 days, 16 to 496 days), 14 cardiovascular deaths occurred and four patients underwent urgent cardiac transplantation, giving a cumulative event rate of 15% for the follow-up period. Of the 14 deaths that occurred, three were due to sudden cardiac death and 11 to progressive heart failure. No patient was lost to follow-up. The main hormonal characteristics in patients with cardiac events or event-free survival were the following: plasma norepinephrine (766 ± 515 vs 534 ± 279 pg . ml^{-1}), plasma atrial natriuretic peptide (406 ± 226 vs 242 ± 217 pg . ml^{-1}), plasma endothelin-1 (5·8 ± 1·4 vs 4·5 ± 1·2 pg . ml^{-1}) and plasma immunoreactive adrenomedullin (714 ± 313 vs 600 ± 287 pg . ml^{-1}). Statistical analysis was not performed on these data because a t-test would not take into account the duration of follow-up. For a similar NYHA class, patients who experienced death or cardiac transplantation tended to have higher concentrations of immunoreactive adrenomedullin than event-free survival patients. These descriptive data are given in Table 2.

Predictors of outcome

Univariate Cox proportional hazard regression analyses showed that plasma levels of immunoreactive adrenomedullin, atrial natriuretic peptide, plasma endothelin-1 and norepinephrine were significant predictors of outcome, with clinical variables such as systolic blood

Table 2 Plasma immunoreactive adrenomedullin concentrations according to NYHA functional class (moderate heart failure vs severe) in patients with cardiac event (death or cardiac transplantation) and in survivors

	Death/transplantation	Survivors	*P*
NYHA Class I/II n=81	714 ± 288 pg . ml^{-1} n=6	599 ± 250 pg . ml^{-1} n=75	0·13
NYHA Class III/IV n=36	714 ± 338 pg . ml^{-1} n=12	603 ± 388 pg ml^{-1} n=24	0·06

Table 3 Univariate and multivariate relation between clinical and biochemical variables and outcome in 117 heart failure patients according to a Cox proportional-hazard model

Variables	Univariate analysis *P*	Multivariate analysis *P*	Risk ratio (IC 95%)	SD of variable
Endothelin-1	0·0001	0·0002	2·56 (1·56, 4·19)	1·3
Heart rate	0·0003	ns		
NYHA functional class	0·0007	ns		
Systolic blood pressure	0·002	0·003	0·35 (0·18, 0·69)	23
Norepinephrine	0·002	ns		
Atrial natriuretic peptide	0·003	ns		
LV ejection fraction	0·003	ns		
ir-adrenomedullin	0·04	0·03	1·56 (1·05, 2·32)	293
LV end-diastolic diameter	0·08	ns		
Age	0·11	ns		
Sex	0·13	ns		

P value based on Cox proportional hazards model.
Risk ratios are calculated for a change in each variable equal to the SD of the variable around its sample mean value.

pressure, heart rate, ejection fraction or NYHA functional class (Table 3). By multivariate, analysis including the variables mentioned above (systolic blood pressure, heart rate, ejection fraction, NYHA functional class, and plasma levels of immunoreactive adrenomedullin, atrial natriuretic peptide, plasma endothelin-1 and norepinephrine), only systolic blood pressure and plasma concentrations of immunoreactive adrenomedullin and plasma endothelin-1 remained significant predictors of outcome (Table 3).The results of the multivariate analysis were similar when age and sex were included in the analysis. When the plasma endothelin-1 plasma levels increased by 1·3 pg . ml^{-1} (SD) the risk increased by 2·5, when the systolic blood pressure increased by 23 mmHg (SD) the risk decreased by 65%, and when the adrenomedullin plasma levels increased by 293 pg . ml^{-1} (SD) the risk increased by 56%.

Discussion

The current findings confirm that the immunoreactive adrenomedullin plasma level is elevated in patients with chronic heart failure treated by ACE inhibitors and diuretics, and indicate for the first time that the plasma concentration of immunoreactive adrenomedullin is a powerful independent predictor of outcome in chronic heart failure. Plasma levels of adrenomedullin have been shown to be predictive of death and heart failure after myocardial infarction, but this relationship did not retain independent significance by multivariate analysis[6]. The discrepancy between this former study[6] and our own may be due to differences in the disease context and the choice of a different end-point. We used a robust end-point of death or urgent cardiac transplantation, whereas the other study used a combined end-point of death and left ventricular failure.

Among established markers of prognosis included in the analysis, immunoreactive adrenomedullin plasma levels brought additional prognostic information to endothelin-1 plasma levels, to assess short-term prognosis of heart failure patients. The addition of new prognostic information given by immunoreactive adrenomedullin plasma determination, although not as powerful as that resulting from endothelin-1 determination, may result from the fact that the two peptides have different functions, and modulation and act through different pathways in the cardiovascular system. Only new studies in large populations will enable comparisons to be made of the respective prognostic

information of the various peptides in chronic heart failure.

Adrenomedullin levels probably reflect a cardiac and systemic response to cardiac impairment and may be mediated by various mechanisms, including increased plasma volume[7,8]. It has been demonstrated that the failing ventricle is the main source of secretion of adrenomedullin[9]. Adrenomedullin is a multifunctional peptide and a potent vasodilating and relaxing peptide. Moreover, adrenomedullin may enhance cardiac contractility via specific receptors and cAMP-independent mechanisms including: Ca^{2+} release from intracellular stores, activation of protein kinase C and Ca^{2+} influx through L-type Ca^{2+} channels[10]. In experimental models, adrenomedullin has been reported to inhibit the secretion of endothelin-1 and angiotensin II, aldosterone and catecholamine[11–13]. Finally, adrenomedullin may also play a role in the kidney by increasing urine sodium excretion and maintaining urine output[14].

Early plasma adrenomedullin is an indicator of left ventricular dysfunction after myocardial infarction[6]. In heart failure, a weak but significant correlation has been reported between ejection fraction and plasma levels of adrenomedullin, but these studies included some patients selected on symptoms and with normal ejection fraction[7,9]. By contrast, we found no relationship between ejection fraction and plasma concentrations of immunoreactive adrenomedullin in patients with reduced left ventricular ejection fraction. Similar results were found in heart failure[15] and in post-myocardial infarction[6]. This lack of relationship suggests that adrenomedullin is not a good marker of the haemodynamic status of chronic heart failure patients.

We found no relationship between immunoreactive adrenomedullin plasma levels and other neurohormone plasma levels, especially endothelin-1 plasma levels. Previous reports indicated that plasma levels of adrenomedullin correlated with plasma levels of atrial natriuretic peptide[7,9]. However, these relationships were weak and appeared mainly restricted to the lower values of the atrial natriuretic peptide plasma levels[9].

Limitations of the study

These results are consistent in the population studied. They should be confirmed in a larger population, including more important subgroups of Class IV chronic heart failure patients. We acknowledge that idiopathic cardiomyopathy was the major aetiology in our study. Although no difference was observed between ischaemic and non-ischaemic patients, it is possible that different results would be observed in a population that included predominantly ischaemic heart failure.

The duration of the study was sufficient to determine prognostic markers of outcome and to assess the short-term prognostic value of adrenomedullin and endothelin-1 plasma levels. However, it would be interesting to study the long-term prognosis of these neurohormones in chronic heart failure.

Our investigation did not include brain natriuretic peptide plasma level measurements. Brain natriuretic peptide, a cardiac natriuretic peptide, is raised in the plasma of patients with left ventricular systolic dysfunction. In cardiac failure, brain natriuretic peptide concentrations provide independent prognostic information[16]. Further studies are necessary to compare the prognostic value of this natriuretic peptide with the prognostic value of endothelin-1 and immunoreactive adrenomedullin.

To summarize, we show here that plasma adrenomedullin is increased in chronic heart failure and represents an independent prognostic factor. This supports a potential role for this peptide in neurohumoral activation in heart failure, but the mechanism of activation of the peptide and the relationship of adrenomedullin with other neurohumoral systems remains unknown. Further studies are necessary to understand the pathophysiological role of the adrenomedullin system in chronic heart failure.

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