

Neurohormones and Cytokines in Heart Failure. Correlation With Coronary Flow Reserve

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Introduction and objectives. In heart failure, the coronary flow reserve (CFR) measured by positron-emission tomography (PET) is reduced. As neurohormone and cytokine levels are also altered in patients with the condition, our aim was to determine whether there is a correlation between CFR and neurohormone and cytokine levels.

Patients and method. The study included 40 patients with heart failure but without ischemic heart disease. Myocardial blood flow was measured by PET using nitrogen-13 ammonia at baseline and during adenosine triphosphate (ATP) infusion. The CFR was calculated for each patient. In addition, levels of the following were determined: norepinephrine, endothelin-1, angiotensin-II, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), tumor necrosis factor- α , interleukin (IL)-1 β , soluble IL-2 receptor, and IL-6.

Results. All factors levels were elevated above reference values. The levels of all cytokines, except IL-1 β , were also elevated. There was a significant negative correlation between CFR and the levels of several neurohormones: ANP ($r=-0.476$), BNP ($r=-0.442$), and IL-6 ($r=-0.509$).

Conclusions. In heart failure, the decrease in CFR is correlated with increases in the levels of certain neurohormones (i.e., ANP and BNP) and cytokines (i.e., IL-6), with vasodilatory effect. These increases are probably compensatory mechanisms insufficient to correct the endothelial dysfunction present in these patients.

Key words: Heart failure. Coronary flow reserve. Neurohormones. Cytokines.

Neurohormonas y citocinas en la insuficiencia cardíaca. Correlación con la reserva de flujo coronario

Introducción y objetivos. La reserva del flujo coronario (RFC) medida con tomografía por emisión de positrones (PET) está disminuida en la insuficiencia cardíaca. Puesto que las neurohormonas y las citocinas también presentan modificaciones en estos pacientes, pretendemos comprobar si hay correlación entre la RFC y los valores de neurohormonas y citocinas.

Pacientes y método. Se estudió a 40 pacientes diagnosticados de cardiopatía no isquémica e insuficiencia cardíaca. El flujo miocárdico (FM) se midió mediante PET y N-13 amonio: en condiciones basales y durante la infusión de trifosfato de adenosina (ATP). En cada uno se calculó la RFC. En todos se determinaron la noradrenalina, la endotelina-1, angiotensina II, el péptido natriurético auricular (ANP) y cerebral (BNP), el factor de necrosis tumoral α , la interleucina (IL)-1 β y el receptor soluble de IL-2 e IL-6.

Resultados. Todas las neurohormonas medidas tuvieron valores superiores a los de referencia. Las citocinas medidas también estuvieron elevadas, excepto la IL-1 β . Se encontró una correlación negativa significativa entre la RFC y los siguientes factores: ANP ($r = -0,476$), BNP ($r = -0,442$) e IL-6 ($r = -0,509$).

Conclusiones. En la insuficiencia cardíaca, la disminución de la RFC se correlaciona con el aumento de determinadas neurohormonas (ANP, BNP) y citocinas (IL-6), de efecto vasodilatador. Se trata, probablemente, de mecanismos de compensación insuficientes ante la disfunción endotelial que presentan estos enfermos.

Palabras clave: Insuficiencia cardíaca. Reserva de flujo coronario. Neurohormonas. Citocinas.

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INTRODUCTION

In a previous study,¹ we demonstrated for the first time that the coronary flow reserve (CFR), measured by positron emission tomography (PET), is reduced in nonischemic heart failure, regardless of the

ABBREVIATIONS

ANP: atrial natriuretic peptide.
BNP: brain natriuretic peptide.
CFR: coronary flow reserve.
IL: interleukin.
PET: positron emission tomography.
TNF- α : tumor necrosis factor-alpha.

etiology. Moreover, in those patients presenting a worse functional class, the reserve is also more markedly affected.

On the other hand, a number of studies show that heart failure is associated with increases in the levels of certain neurohormones and cytokines,²⁻¹¹ including norepinephrine, angiotensin II, endothelin 1, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), interleukin (IL)-1, IL-6, soluble IL-2 receptor, and tumor necrosis factor-alpha (TNF- α). The extent of these increases is generally related to the prognosis.

The objective of this study was to establish the possible correlation between PET-measured CFR and neurohormone and cytokine levels.

PATIENTS AND METHODS

Patients

The study included 40 consecutive patients (mean age, 63 $\pm$ 11 years; 27 men), 30 outpatients and 10 inpatients being treated in the cardiology service. The inclusion criteria were age over 18 years, nonischemic heart disease and a history of heart failure. Those patients presenting infection, chronic inflammatory disease, malignant tumor and/or renal failure (serum creatinine greater than 2 mg/dL) were excluded, as were women who might be pregnant. The study was approved by the ethics committee of the center and all the patients signed an informed consent form prior to their inclusion.

Studies

All the patients were assessed on the basis of a complete clinical history, physical examination, routine analyses (differential cell count, systematized urinalysis and blood chemistry), 12-lead electrocardiogram and

echocardiogram. All of them had undergone coronary angiography within the preceding 6 months.

Treatment

At the time of the study, the patients were being treated with angiotensin-converting enzyme inhibitors, angiotensin II receptors, digitalis, diuretics and beta-blockers. Each patient was taking several of these drugs and some patients were receiving all of them.

Positron Emission Tomography

Eighty studies were performed in the 40 patients. In each, PET was carried out with ¹³N ammonium at baseline (resting) and immediately after infusion of adenosine triphosphate to induce stress.¹

Neurohormones and Cytokines

Venous blood samples were taken after an overnight fast and 30 minutes of rest in a supine position. All the collection tubes contained the anticoagulant EDTA. The samples were centrifuged for 30 minutes and the plasma stored at -80°C for subsequent analysis.

The neurohormones were determined by radioimmunoassay according to the instructions of the manufacturer (IRMA, CIS bio international, France). The normal values for our laboratory, established previously with a control group, are: norepinephrine, 273 $\pm$ 102 pg/mL; endothelin-1, 1.76 $\pm$ 0.8 pg/mL; angiotensin II, 6.1 $\pm$ 3 pg/mL; ANP, 19 $\pm$ 5 pg/mL and BNP, 17.4 $\pm$ 4.8 pg/mL. The cytokines were determined by enzyme immunoassay following the instructions of the manufacturer (RD System, Abingdon, UK). The normal values are: TNF- α , 3.1 $\pm$ 0.6 pg/mL; IL-1 β , 0.5 $\pm$ 0.4 pg/mL; soluble IL-2 receptor, 1007 $\pm$ 250 pg/mL, and IL-6, 1.77 $\pm$ 0.9 pg/mL.

Statistical Analysis

The study data were loaded on a SPSS database. The descriptive statistics were the mean and the standard deviation. The correlation between the quantitative variables was examined using Pearson's coefficient when the distribution was normal and the Spearman coefficient when it was not. A *P* value of less than .05 was considered to indicate statistical significance.

RESULTS

Characteristics of the Patients Studied

Table 1 shows the demographic characteristics and cardiovascular risk factors of the patients enrolled in

TABLE 1. Patient Characteristics*

Number of patients	40
Age, mean (SD), years	63±11
Men, n (%)	27 (68)
BMI, mean (SD)	27.7±4.4
Smokers, n (%)	5 (12.5)
Hypertension, n (%)	24 (60)
Diabetes mellitus, n (%)	6 (15)
TC/HDL-C, mean (SD)	5.75±1.37

*BMI indicates body mass index; SD, standard deviation; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol.

TABLE 2. Echocardiographic Diagnosis and Variables*

	Number
Dilated cardiomyopathy, n (%)	24 (60)
Mitral-aortic valve disease, n (%)	15 (37.5)
Restrictive cardiomyopathy, n (%)	1 (2.5)
Ejection fraction, mean (SD), %	41±15
LV end-diastolic diameter, mean (SD), mm	62±13
LV EDV index, mean (SD), ml/m ²	113±53

*EDV indicates end-diastolic volume; LV, left ventricular; SD, standard deviation.

TABLE 3. Neurohormone Levels*

Neurohormone	Patients	Reference Value
ET-1, mean (SD), pg/mL	19±26	1.76±0.8
AG-II, mean (SD), pg/mL	27±38	6.1±3
ANP, mean (SD), pg/mL	74±86	19±5
BNP, mean (SD), pg/mL	137±210	17.4±4.8

*AG-II indicates angiotensin II; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ET-1, endothelin-1.

the study. The diagnoses and major echocardiographic variables appear in Table 2.

Neurohormones and Cytokines

The mean values of the neurohormones and cytokines measured are displayed in Tables 3 and 4, respectively. Table 3 shows that the levels of all the neurohormones measured were elevated with respect to the reference values, while the results in Table 4 demonstrate that all the cytokines measured, except for IL-1 β , were also higher than their reference values.

Coronary Flow Reserve Measured by Positron Emission Tomography

These values are summarized in Table 5. Taking into account the fact that the normal CFR in individuals of an age similar to that of the patients included in this study is 3±0.73,¹² this variable is seen to be considerably reduced in the study population.

TABLE 4. Cytokine Levels*

Cytokine	Patients	Reference Value
TNF- α , mean (SD), pg/mL	24.4±1.99	3.1±0.6
IL-1 β , mean (SD), pg/mL	0.34±0.56	0.5±0.4
sIL-2R, mean (SD), pg/mL	1647±799	1007±250
IL-6, mean (SD), pg/mL	24.6±3.2	1.77±0.9

*IL-1 β indicates interleukin 1 β ; IL-6, interleukin-6; sIL-2R, soluble interleukin-2 receptor; SD, standard deviation; TNF- α , tumor necrosis factor alpha.

TABLE 5. Myocardial Flow and Coronary Flow Reserve Measured by Positron Emission Tomography*

MF _b , mean (SD), mL.g ⁻¹ .min ⁻¹	0.76±0.26
MF _{ATP} , mean (SD), mL.g ⁻¹ .min ⁻¹	1.43±0.77
CFR (MF _{ATP} /MF _b), mean (SD)	1.87±0.88

*ATP indicates adenosine triphosphate; b, baseline; CFR, coronary flow reserve; MF, myocardial flow.

TABLE 6. Correlation Between Reserve and Neurohormones and Cytokines*

	Correlation Coefficient	P
ET-1	-0.100	.551
AG-II	-0.045	.790
ANP	-0.476	.003
BNP	-0.442	.005
TNF- α	0.73	.678
IL-1 β	0.009	.960
sIL-2R	-0.97	.580
IL-6	-0.509	.002

*AG-II indicates angiotensin II; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; ET-1, endothelin-1; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; sIL-2R, soluble interleukin-2 receptor; TNF- α , tumor necrosis factor alpha.

Correlation Between Coronary Flow Reserve and Neurohormone and Cytokine Levels

As can be seen in Table 6, there was a significant negative correlation between CFR and ANP, BNP, and IL-6. No correlation was found with the remaining variables.

DISCUSSION

Neurohormones

The present study demonstrates that heart failure is associated with neurohormonal activation, with increases in all the variables measured with respect to the reference values. Norepinephrine showed the least change, a fact that might be attributable to the drugs taken by the patients, some of which (the beta-blockers) inhibit the sympathetic nervous system.

Angiotensin II is increased despite the fact that most of the patients were being treated with angiotensin-converting enzyme receptors or ARBs. Without these drugs, the increase would probably have been greater.

Cytokines

Among the cytokines determined in the patients, TNF- α , IL-6 and soluble IL-2 receptor were higher than their reference values. These results agree with those reported by most authors, who have found heart failure to be associated with neurohormonal activation and elevated cytokine concentrations.²⁻¹¹

However, there was no increase in IL-1 β . This cytokine plays an important pathophysiological role in the origin and development of heart failure but, in contrast to other cytokines, its actions are focused basically on the myocardium.¹³ This fact would explain the failure of the majority of the studies,¹⁴ including ours, to demonstrate elevated IL-1 β values in peripheral blood.

IL-6 is the cytokine that showed the most marked increase in our study, a circumstance that agrees with a large number of reports that demonstrate a fundamental role for IL-6 in heart failure.^{2,9,15,16}

Coronary Flow Reserve Measured by Positron Emission Tomography in Heart Failure

Patients with heart failure in the absence of ischemic heart disease have reduced CFR. The most marked decrease is detected in individuals in New York Heart Association functional class III-IV,¹ who present significant differences with respect to those who are in functional class I. In this study, we present the overall mean values for the total group of 40 patients, without dividing them into subgroups, in order to simplify the data.

Correlation Between Coronary Flow Reserve, Neurohormones, and Cytokines

The present study demonstrates a significant negative correlation between PET-measured CFR and certain variables (ANP, BNP, and IL-6) in patients with nonischemic heart failure. We expected to observe a negative correlation between the CFR and those neurohormones and cytokines, such as norepinephrine, angiotensin II, endothelin-1, and TNF- α , that produce vasoconstriction, but this was not the case.

Natriuretic peptides are known to have both arterial and venous vasodilatory properties and, thus, they exert a compensatory effect in heart failure.¹⁷ IL-6 enhances endothelium-dependent vasodilation, while it induces myocardial dysfunction. This suggests that its local production regulates resistance and vascular permeability in these patients.¹⁸ It is a well-known fact

that heart failure is associated with endothelial dysfunction,¹⁹ with vasoconstriction of large and small vessels of the myocardium²⁰ and the peripheral arteries.²¹ This may be due not only to sympathetic activation and increases in endothelin-1 and TNF- α , but to other mechanisms, such as the augmented production of oxygen free radicals.^{19,22,23} The increases in ANP, BNP and IL-6 may be compensatory mechanisms to counteract the vasoconstriction owing to a number of causes.

To the best of our knowledge, the correlation between PET-measured CFR and neurohormone and cytokine levels in heart failure has not been evaluated previously. As is the case in so many processes, some compensatory mechanisms prove to be deleterious; one such mechanism is the increase in IL-6, which can intensify the severity of ventricular dysfunction and, thus, of heart failure.

Limitations

The major limitation of this study is the small number of patients included, owing to the difficulty associated with PET, which is a complex and costly technique. Thus, it can not be performed in as many patients as would be desirable. The data presented here do not allow to know whether or not the evaluation of a greater number of cases would enable the detection of a greater number of significant relationships than those observed in this study.

CONCLUSIONS

The reduction of the CFR observed in heart failure correlates with increases in certain neurohormones (ANP and BNP) and cytokines (IL-6), all of which have vasodilatory effects. This circumstance suggests that they are probably involved in compensatory mechanisms that prove to be insufficient against the multifactorial endothelial dysfunction presented by these patients.

REFERENCES

1. Coma-Canella I, García-Velloso MJ, Macías A, Villar L, Cosín-Sales J, Martí-Climent JM, et al. Disminución de la reserva de flujo coronario en pacientes con insuficiencia cardíaca no isquémica. *Rev Esp Cardiol*. 2003;56:354-40.
2. Vidal B, Roig E, Pérez-Villa F, Orús J, Pérez J, Jiménez V, et al. Valor pronóstico de los niveles de citocinas y neurohormonas en la insuficiencia cardíaca severa. *Rev Esp Cardiol*. 2002;55:481-6.
3. Francis GS, Cohn JN, Johnson G, Rector TS, Goldman S, Simon A. Plasma noradrenaline, plasma renin activity and congestive heart failure: relations to survival and the effects of therapy in V-HeFT II. *Circulation*. 1993;87 Suppl VI:40-8.

4. Francis G. The relationship of the sympathetic nervous system and the renin-angiotensin system in congestive heart failure. *Am Heart J*. 1989;118:642-8.
5. Wei CM, Lerman A, Rodeheffer RJ, McGregor CG, Brandt RR, Wright S, et al. Endothelin in human congestive heart failure. *Circulation*. 1994;89:1580-6.
6. Winters TJ, Sallman AL, Baker BJ, Meadows J, Rico DM, Vesely DL. The N-terminus and 4000 molecular weight peptide from the mid portion of the N-terminus of the atrial natriuretic factor prohormone each circulate in humans and increase in congestive heart failure. *Circulation*. 1989;80:438-49.
7. Davis M, Espiner E, Richards G, Bilings J, Town I, Neil A. Plasma brain natriuretic peptide in assesment of acute dyspnoea. *Lancet*. 1994;343:440-4.
8. Testa M, Yeh M, Lee P, Fanelli R, Loperfido F, Berman JW, et al. Circulating levels of cytokines and their endogenous modulators in patient with mild to severe congestive heart failure due to coronary artery disease or hypertension. *J Am Coll Cardiol*. 1996;28:964-71.
9. Roig E, Orus J, Pare C, Azqueta M, Filella X, Pérez-Villa F, et al. Serum interleukin-6 in congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1998;82:688-90.
10. Limas CJ, Goldenberg IF, Limas C. Soluble interleukin-2 receptor in patients with dilated cardiomyopathy. Correlation with disease severity and cardiac autoantibodies. *Circulation*. 1995;91:631-4.
11. Bristow MR. Tumor necrosis factor-alpha and cardiomyopathy. *Circulation*. 1998;97:1340-1.
12. Czernin J, Muller P, Chan S, Brunken RC, Porenta G, Krivokapich J, et al. Influence of age and hemodynamics on myocardial blood flow and flow reserve. *Circulation*. 1993;88:62-9.
13. Baumgarten G, Knuefermann P, Mann DL. Cytokines as emerging targets in the treatment of heart failure. *Trends Cardiovasc Med*. 2000;10:216-23.
14. Aukrust P, Ueland T, Lien E, Bendtzen K, Muller F, Andreassen AK, et al. Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol*. 1999;83:376-82.
15. Suzuki H, Sato R, Sato T, Shoji M, Iso Y, Kondo T, et al. Time-course of changes in the levels of interleukin 6 in acutely decompensated heart failure. *Int J Cardiol*. 2005;100:415-20.
16. Hirota H, Izumi M, Hamaguchi T, Sugiyama S, Murakami E, Kunisada K, et al. Circulating interleukin-6 family cytokines and their receptors in patients with congestive heart failure. *Heart Vessels*. 2004;19:237-41.
17. Abassi Z, Karram T, Ellaham S, Winaver J, Hoffman A. Implications of the natriuretic peptide system in the pathogenesis of heart failure: diagnostic and therapeutic importance. *Pharmacol Ther*. 2004;102:223-41.
18. Tsutamoto T, Hisanaga T, Wada A, Maeda K, Ohnishi M, Fukai D, et al. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. *J Am Coll Cardiol*. 1998;31:391-8.
19. Fang ZY, Marwick TH. Vascular dysfunction and heart failure: epiphenomenon or etiologic agent? *Am Heart J*. 2002;143:383-90.
20. Mathier MA, Rose GA, Fifer MA, Miyamoto MI, Dinsmore RE, Castano HH, et al. Coronary endothelial dysfunction in patients with acute-onset idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*. 1998;32:216-24.
21. Katz SD. The role of endothelium-derived vasoactive substances in the pathophysiology of exercise intolerance in patients with congestive heart failure. *Prog Cardiovasc Dis*. 1995;26 Suppl 2:9-12.
22. Sharma R, Davidoff MN. Oxidative stress and endothelial dysfunction in heart failure. *Congest Heart Fail*. 2002;8:165-72.
23. Bauersachs J, Schafer A. Endothelial dysfunction in heart failure: mechanisms and therapeutic approaches. *Curr Vasc Pharmacol*. 2004;2:115-24.