ORIGINAL ARTICLES

▌Heart Failure

Neurohormonal Activation in Congestive Heart Failure: Does it Normalize After Heart Transplantation?

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Introduction and objective. In patients with congestive heart failure, neurohormonal activation plays an important role in disease progression and prognosis. The aim of this study was to document the evolution of neurohormonal activation after heart transplantation.

Patients and method. Thirty-seven patients on the waiting list for heart transplantation were included in the study. Plasma levels of angiotensin II, aldosterone, endothelin, atrial natriuretic peptide and adrenomedullin were measured before heart transplantation and again 1, 4, 9 and 12 months afterwards. Plasma levels of norepinephrine and renin were measured before and 1 month after heart transplantation.

Results. The levels of angiotensin II, norepinephrine and renin showed a nonsignificant trend towards reduction. The levels of aldosterone were unchanged, and an increase in endothelin levels was seen 9 and 12 months after transplantation. Plasma levels of atrial natriuretic peptide and adrenomedullin were significantly lower 1, 4, 9 and 12 months after heart transplantation compared to pretransplant levels.

Conclusions. During the first several months after heart transplantation there were no significant reductions in plasma levels of angiotensin II, aldosterone and endothelin, and there were significant reductions soon after surgery in peptides with a predominantly vasodilator effect (atrial natriuretic peptide and adrenomedullin). This unfavorable neurohormonal profile may contribute to the development of posttransplant complications such as edema, arterial hypertension and endothelial dysfunction.

Key words: *Neurohormones. Heart failure. Heart transplantation.*

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Activación neurohormonal en la insuficiencia cardíaca congestiva: ¿se normaliza después del trasplante cardíaco?

Introducción y objetivo. En los pacientes con insuficiencia cardíaca congestiva, la activación neurohormonal desempeña un papel importante en la progresión de la enfermedad y en el pronóstico. El objetivo de este estudio fue determinar la evolución de la activación neurohormonal después del trasplante cardíaco.

Pacientes y método. Se incluyó en el estudio a 37 pacientes en lista de espera para trasplante cardíaco. Se determinaron las concentraciones plasmáticas de angiotensina II, aldosterona, endotelina, péptido natriurético auricular (PNA) y adrenomedulina antes y 1, 4, 9 y 12 meses después del trasplante cardíaco. Las concentraciones plasmáticas de noradrenalina y renina se determinaron antes y 1 mes después del trasplante.

Resultados. Las concentraciones de angiotensina II, noradrenalina y renina mostraron una tendencia no significativa hacia la reducción. Las concentraciones de aldosterona no se modificaron, mientras que se produjo un incremento en las de endotelina a los 9 y 12 meses tras el trasplante. Las concentraciones plasmáticas de PNA y de adrenomedulina disminuyeron significativamente 1, 4, 9 y 12 meses después del trasplante, comparadas con los valores previos al trasplante.

Conclusiones. Durante los primeros meses posteriores al trasplante cardíaco, no se producen reducciones significativas en las concentraciones plasmáticas de angiotensina II, aldosterona y endotelina, mientras que se reducen significativamente las concentraciones de péptidos con un efecto predominante vasodilatador (PNA y adrenomedulina). Este perfil neurohormonal desfavorable podría contribuir al desarrollo de complicaciones postrasplante, tales como edemas, hipertensión arterial y disfunción endotelial.

Palabras clave: *Neurohormonas. Insuficiencia cardíaca. Trasplante.*

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INTRODUCTION

Neurohormonal activation plays an important role in disease progression and prognosis in patients with congestive heart failure. Treatments with agents that block the effect of circulating neurohormones consequently reduce morbidity and mortality associated with the disease.[1-9]

A gradual normalization of neurohormonal activity may occur following heart transplant, but the temporal evolution of this has never been studied prospectively. After years of maintaining high concentrations of circulating neurohormones, it would seem reasonable that a return to normal levels should take several weeks or even months. If this is the case, high concentrations of vasoactive hormones during the post-operative period following heart transplant could have a negative influence on patient outcomes, especially common complications as right ventricular dysfunction, pulmonary hypertension, acute kidney failure or edema.

In addition, treatment with inhibitors of angiotensin converting enzyme (ACE inhibitors), beta-adrenergic blockers and anti-aldosterone agents is usually abruptly interrupted at the time of transplant, and is never systematically reintroduced.

The aim of the present study was to analyze prospectively and systematically changes in concentrations of circulating vasoactive hormones after heart transplant in patients treated for terminal heart failure caused by systolic dysfunction.

PATIENTS AND METHODS

Study Population

All patients with terminal heart failure due to systolic dysfunction who received a heart transplant in the Hospital Clínic of Barcelona between June 2000 and December 2001 were candidates for inclusion in the study. The exclusion criteria were acute heart failure as an indication for transplant, and non-availability of post-transplant analysis results due to death during the immediate post-operative period.

All patients underwent routine examinations before and after transplant, according to the established protocol of our hospital. All patients received isoproterenol and dobutamine during the first week after surgery. Induction therapy with muromonab-CD3 (ORTHOCLONE OKT3, Ortho Biotech Inc, New Jersey) was provided for the first 5 days after transplant. All patients received immunosuppressive treatment with cyclosporin (initiated on the third day post-transplant and adjusted to maintain values of 250-350 nmol/L for the first 3 months, 150-250 nmol/L thereafter) or tacrolimus (10-15 nmol/L for the first 3 months, 7-10 nmol/L thereafter), azathioprine (initial dose 2 mg/kg/day, adjusted to maintain leukocytes above 4000 per mL) or mycophenolate mofetil (initial dose 2 g/day, adjusted to maintain concentrations of 2-4 µg/mL), and corticoids. High blood pressure was treated with calcium channel antagonists (amlodipine or diltiazem). When high blood pressure persisted, an angiotensin receptor antagonist (losartan) or an ACE inhibitor was also prescribed. Anti-hypertensive medication was maintained until the day before blood analysis.

To avoid the need for extra venous punctures, blood was extracted for both the study and for routine analysis at the same time.

The ethics committee of our center approved the protocol and all patients gave their informed consent to be included.

Study Protocol

Plasma concentrations of angiotensin II, aldosterone, endothelin, atrial natriuretic peptide (ANP), and adrenomedullin were determined before heart transplant and then again at 1, 4, 9, and 12 months post-transplant. Plasma renin activity and norepinephrine concentrations were also measured before surgery and then again at 1 month after surgery.

An antecubital vein was used for the collection of blood, fasted and bed-rested (2 h) patients. Blood was extracted after 45 min to measure the activity of plasma renin and to determine the plasma concentrations of angiotensin II, aldosterone, norepinephrine, endothelin, ANP, and adrenomedullin. All samples were maintained on ice, as previously described.[8] Tubes were centrifuged at -4°C and the plasma frozen at -30°C until analysis. The values recognized as normal by our laboratory are: angiotensin II (<16 pg/mL; range, 5-15 pg/mL), aldosterone (<30 ng/mL; range, 4-30 ng/mL), endothelin (<12 pmol/L), ANP (6 ± 1 fmol/mL), adrenomedullin (127 ± 13 pg/mL), norepinephrine (235 ± 114 pg/mL; range, 143-407 pg/mL^{-1}), and plasma renin activity (1.4 ± 0.9 ng/mL/h; range, 0.2-2.9 ng/mL/h).

Statistical Analysis

Results are expressed as means ± standard deviation. The Student t test was used to compare pre- and post-transplant values for paired samples. The Wilcoxon test was used when appropriate. Significance was set at $P<.05$.

RESULTS

Of the 41 patients considered for the study, 2 were excluded since they died immediately after surgery. Another 2 were excluded because of the acute etiology of the disease leading to their heart transplant (acute myocardial infarction with cardiogenic shock). The remaining 37 composed the study population. Table 1 shows the pre-transplant characteristics of these patients.

After surgery, 80% of the patients received cyclosporin, 20% received tacrolimus, 71% received mycophenolate mofetil, and 29% received azathioprine. Eighty four percent received calcium channel antagonists and 11% received angiotensin receptor inhibitors.

The angiotensin II concentrations recorded showed a non-significant downward trend in the analyses made at 1 and 4 months post-transplant. Afterwards, however, they increased slightly (Figure 1). The differences were not statistically significant probably because of the wide standard deviations associated

Fig. 1. Plasma concentration of angiotensin II (mean±SD) before (0) and 1, 4, 9, and 12 months after heart transplant. The dotted line represents the upper limit of variation for 95% of the normal values in healthy subjects.

with the values recorded. It is noteworthy that, before transplant, 78% of patients had angiotensin II concentrations above the normal limit. This percentage diminished to 58% 1 month after transplant, to 50% at 4 months, to 46% at 9 months, and was 53% at 12 months.

The concentration of aldosterone did not change over the experimental period (Figure 2); endothelin levels, however, were significantly higher at 9 and 12 months post-transplant (Figure 3).

As expected, both the norepinephrine concentrations and plasma renin activity showed a tendency to diminish after surgery (norepinephrine: 335±235 pg/mL before and 239±135 pg/mL after surgery, plasma renin activity: 7.7±8 ng/mL/h before and 5.3±8 ng/mL/h after surgery).

Plasma ANP concentrations were significantly lower after surgery, and remained low in all post-transplant analyses (Figure 4). The adrenomedullin concentrations, however, showed a non-significant increase in the first month post-transplant, followed by a significant decline in the following months. Normal values were observed in 78% of patients at 12 months (Figure 5).

No differences were seen in terms of the neurohormonal profile between the patients treated with cyclosporin and those treated with tacrolimus, nor between those who received azathioprine or mycophenolate mofetil. Medication for hypertension was adjusted over the course of the study, but no alterations in neurohormone levels associated with this were recorded.

No patient died during the year of follow-up. The mean number of episodes of acute cellular rejection (grade ≥3A on the International Society for Heart and Lung Transplantation scale) was 1.3 per patient. None

TABLE 1. **Pre-Transplant Characteristics of Patients***

Age, years±SD (range)	54±8 (30-68)
Men/women	28 (76%)/9 (24%)
Etiology	
Ischemic	14 (38%)
IDCM	15 (41%)
Valvular	8 (21%)
NYHA functional class	
III	27 (73%)
IV	10 (27%)
Ejection fraction, %	21±4
EDDLV, mm	73±9
EDSLV, mm	61±9
Medication	
ACE inhibitors	27 (73%)
ARA-II	7 (19%)
Beta-blockers	14 (38%)
Spironolactone	26 (70%)
Digoxin	26 (70%)
Furosemide	37 (100%)
Dobutamine	8 (22%)

*ARA-II indicates angiotensin II receptor antagonist; EDDLV, end-diastolic diameter of the left ventricle; EDSLV, end-systolic diameter of the left ventricle; ACE inhibitor, angiotensin converting enzyme inhibitor; IDCM, idiopathic dilated cardiomyopathy; NYHA, New York Heart Association.
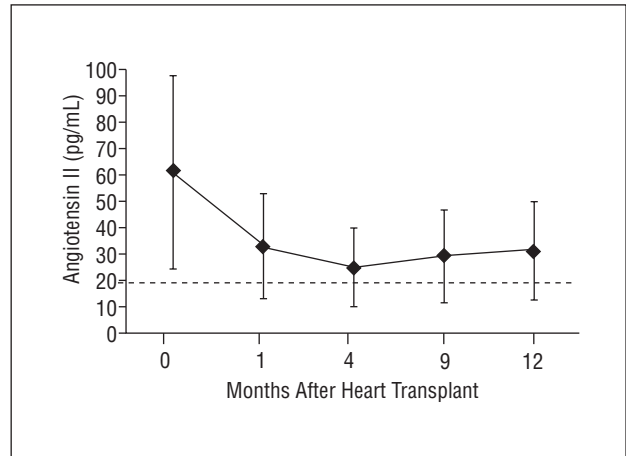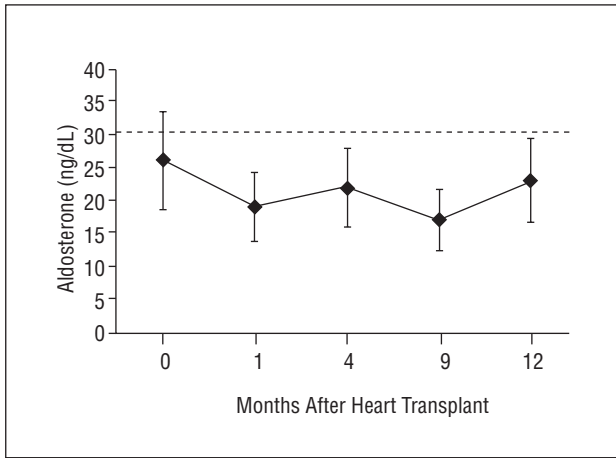
Fig. 2. Plasma concentration of aldosterone (mean±SD) before (0) and 1, 4, 9, and 12 months after heart transplant. The dotted line represents the upper limit of variation for 95% of the normal values for healthy subjects.

Fig. 4. Plasma concentration of atrial natriuretic peptide (mean±SD) before (0) and 1, 4, 9, and 12 months after heart transplant. The dotted line represents the upper limit of variation for 95% of the norm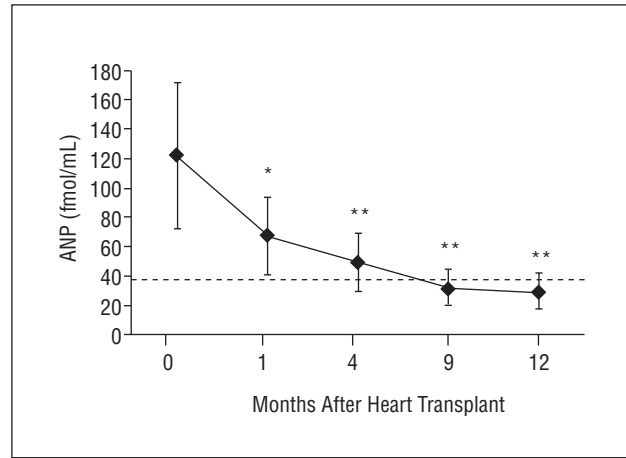al values for healthy subjects. *$P<.05$ compared to pre-transplant values **$P<.001$ compared to pre-transplant values.
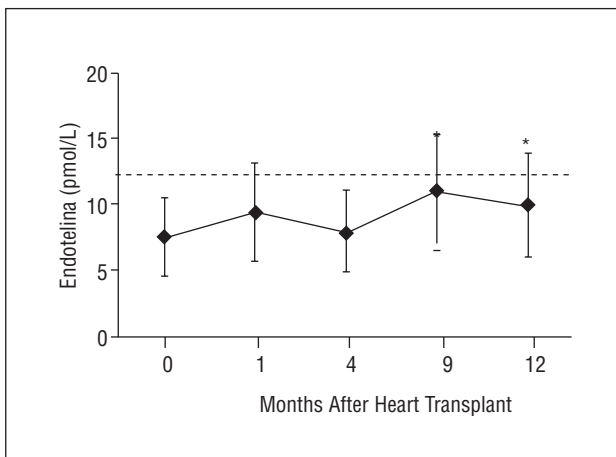

Fig. 3. Plasma concentration of endothelin (mean±SD) before (0) and 1, 4, 9, and 12 months after heart transplant. The dotted line represents the upper limit of variation for 95% of the normal values for healthy subjects. *$P<.01$ compared to pre-transplant values.

Fig. 5. Change in plasma concentration of adrenomedullin (mean±SD) before (0) and 1, 4, 9, and 12 months after heart transplant. The dotted line represents the upper limit of variation for 95% of the normal values for healthy subjects. *$P<.01$ compared to pre-transplant values.
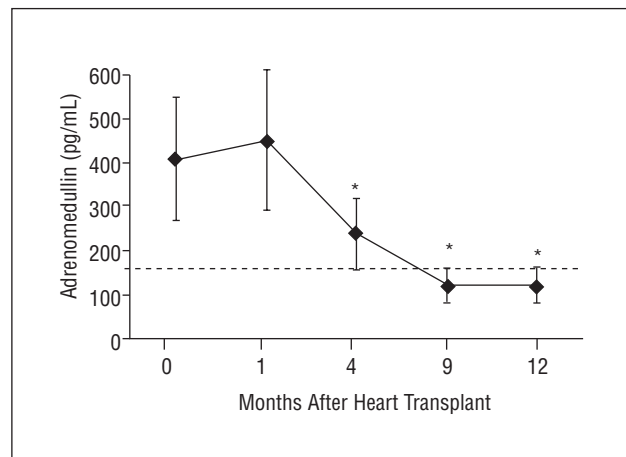
of these episodes was accompanied by echocardiographic signs nor clinical symptoms. All patients were treated with intravenous methylprednislone (1 g for 3 consecutive days). Since blood extractions were made before the corresponding biopsies, they were not affected by the treatment for rejection. This was administered later (normally one day after the biopsy). No differences in neurohormonal levels were seen between episodes of rejection and non-rejection.

DISCUSSION

The present study shows that, during the months following heart transplant, there is a tendency towards a normalization of neurohormonal activity. Nevertheless, while this normalization was very evident with respect to the vasodilatory peptides (adrenomedullin and ANP), it was much less pronounced for those with vasoconstrictory activity (especially angiotensin II), whose concentrations remained high in 50% of patients one year after surgery.

The question arises as to whether these findings are related to a non-specific response to cardiac surgery with extracorporeal circulation. Taylor et al[10] reported on plasma angiotensin II levels in patients who underwent heart surgery with and without such

circulation. Angiotensin II levels increased slightly in both groups of patients following sternotomy. In the control group (closed mitral valvulotomy), no further increase in angiotensin II concentration was recorded, whereas in the extracorporeal circulation group a marked increase was seen, which normalized completely between 4 and 24 h after surgery. Boldt et al[11] measured endothelin, ANP, epinephrine, and norepinephrine concentrations as well as ACE activity in 28 patients that underwent coronary revascularization, 14 of which received intravenous enalapril before surgery. The ACE activity of these patients did not change during surgery, but their concentrations of endothelin, ANP, epinephrine and norepinephrine increased, with a tendency to norma-lize at the end of the operation (no later measurements were made). These changes were less evident in the patients that received ACE inhibitors. Overall, there appears to be no evidence that heart surgery *per se* should justify a significant increase in neurohormonal activation more than 24-48 h after the procedure.

The fact that more than half of the patients from the present study had abnormally high angiotensin II concentrations during the first year after transplant is surprising. This is the first report of this kind, and could have clinical implications. Angiotensin II concentrations similar to those of the present transplant patients are considered a marker of poor prognosis in heart failure, and are associated with a deterioration in ventricular function and an increase in mortality.[12] It is true that only a small fraction of the present patients received ACE inhibitors or angiotensin receptor blockers. This reflects the common practice of trying to avoid the use of these agents in transplant patients, especially just after surgery, as they might cause a deterioration in renal function. Treating the present patients with diuretics (100% the first month, 56% by the end of the first year) might have contributed to the activation of the renin-angiotensin-aldosterone (RAA) system.[13,14] Nonetheless, the use of diuretics in the post-transplant patient is now general practice, and for the time being there is no better alternative from a clinical point of view. In an interesting study of 11 heart transplant patients for whom a 7-41 month post-transplant time had elapsed, Braith et al[15] observed that the baseline values of renin activity were significantly higher than those of controls, and that these patients' angiotensin II concentrations showed a non-significant tendency to be higher. These authors suggested cardiac denervation as another possible cause of the activation of the RAA system.

In the present study, the concentration of endothelin (another potent vasoconstrictory peptide) increased significantly by 9 and 12 months post-transplant. This finding, which confirms that reported by other authors, has been attributed to an increase in the release of endothelin caused by the presence of high concentrations of circulating cytokines (tumor necrosis factor, interleukin 6) and to treatment with anticalcineurinic agents.[16-18]

In contrast with angiotensin II and endothelin, vasodilatory peptides showed a tendency to rapidly normalize after transplant surgery. Previous studies have reported high plasma ANP and brain natriuretic peptide levels following heart transplant which were related to inflammatory activity in the allograft.[19] However, in agreement with the findings from our study, although circulating concentrations of ANP remained initially high after transplant, they then significantly decreased to become similar to those of the normal population at 9 months.

With respect to adrenomedullin levels, Geny et al[20] reported increased plasma concentrations of this vasodilatory peptide during the first month after transplant. However, in another group of nine patients who had undergone transplant 32±16 months earlier, adrenomedullin levels were still higher than those of normal controls. The present study, which included more patients, shows that despite a tendency for adrenomedullin concentrations to increase during the first month after surgery, there is a rapid decline in the following months, such that at 9 months the majority of patients have concentrations within the normal range.

CLINICAL IMPLICATIONS

Overall, the neurohormonal activation pattern in transplanted patients is similar to, or even more unfavorable than, that recorded before heart transplant. As in patients with heart failure, the post-transplant scenario with respect to circulating vasoactive peptides is characterized by a tendency towards hydrosaline retention and vasoconstriction. It is therefore reasonable to assume that at least some of the symptoms associated with neurohormonal activation will persist as well. It is also very probable, therefore, that this activation is involved in the appearance of edemas, in the tendency towards high blood pressure, in the persistence of pulmonary hypertension, and in endothelial dysfunction after transplant. Other factors are also doubtlessly involved in each of these complications.[21-23] The appearance of edemas in the first weeks following heart transplant are traditionally attributed to the persistence of pulmonary hypertension, dysfunction of the right ventricle, renal dysfunction, and high doses of corticoids. Also, the persistent activation of the RAA system should be added to this list. Similarly, the appearance of high blood pressure following heart transplant, typically associated with anticalcineurinic treatment and high concentrations of endothelin, is also favored by denervation of the transplanted organ and the ensuing

loss of neuroendocrine cardio-renal reflexes. Such a loss could, in turn, favor an increase in the extracellular volume, in high blood pressure, and in the stimulation of the RAA system.[15,24-27]

The association between neurohormonal activation and endothelial dysfunction, both coronary and peripheral, is of particular interest. It is well known that, in non-transplanted patients, high concentrations of angiotensin II are associated with endothelial dysfunction of the coronary and peripheral arteries.[28,29] In transplanted patients, endothelial dysfunction of the coronary arteries of the graft is well documented, and has been associated with the later development of vascular disease of this organ.[30] Several factors are again involved in the appearance of coronary endothelial dysfunction (ischemic lesions sustained during transplant surgery, immunological lesions related to rejection of the graft, infection by cytomegalovirus, etc), but there can be no doubt that high angiotensin II levels must also be taken into account. This is supported by the fact that transplanted patients also show endothelial dysfunction in peripheral arteries.[31] Further, in experimental models, treatment with ACE inhibitors and angiotensin receptor blockers reduces the appearance of vascular disease in the graft.[32,33] The finding that high post-transplant angiotensin II levels are associated with the later appearance of coronary artery disease in the graft is of enormous interest; this needs to be confirmed, however, with larger populations and over longer follow-up times.

The aim of the present study was to show that, following heart transplant, significant neurohormonal activation persists, and not to explain the association between neurohormonal activation and clinical progress, for which a much larger sample is warranted. However, the results are very interesting, and could form the initial basis for justifying the early, systemic administration of ACE inhibitors or angiotensin receptor blockers following heart transplant.

In conclusion, in the months following heart transplant, there is a tendency for neurohormonal activation to normalize. This is very evident with respect to the concentrations of vasodilatory peptides (adrenomedullin and ANP), but less so with respect to vasoconstrictory peptides, especially angiotensin II. Concentrations of the latter remained high in half of all patients one year after transplant. This unfavorable neurohormonal profile could contribute to the appearance of complications such as edemas, high blood pressure and endothelial dysfunction.

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