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Plasma Levels of Adrenomedullin, a Newly Identified Hypotensive Peptide, in Patients with Orthotopic Heart Transplantation

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The present study was designed to measure plasma levels of the newly identified vasorelaxant peptide adrenomedullin (AM) in heart transplanted patients and to investigate the hypothetical relationships between plasma AM and some haemodynamic and humoral parameters.

Plasma AM was measured in 16 patients who underwent an orthotopic heart transplantation more than 6 months before. Eleven out of 16 (68.7 %) patients after cardiac transplantation were hypertensives. The control group included 21 normotensive healthy subjects and 15 patients with essential hypertension (WHO stage 1). In all transplanted patients mean AM plasma level was 23.7 ± 8.6 pg/ml, 73 % higher than in normotensive control subjects (13.7 ± 6.1 pg/ml, $p < 0.05$). In patients with essential hypertension AM plasma levels were higher (22.7 ± 8.2 pg/ml) with respect to normal subjects ($p < 0.05$), but the difference was not statistically significant ($p > 0.05$) if compared with that measured in transplanted patients. In this series of cardiac transplant recipients AM plasma levels were 24 % higher in hypertensive transplant recipients (25.5 ± 9.7 pg/ml) than in normotensive transplant recipients (20.5 ± 4.4 pg/ml, $p < 0.05$).

AM plasma levels did not correlate with blood pressure, heart rate or cyclosporine plasma levels.

The present study shows that AM plasma levels were significantly higher in cardiac transplant recipients and in particular in those with hypertension. *J Clin Basic Cardiol 2000; 3: 177–80.*

Key words: adrenomedullin, heart transplantation, hypertension

Adrenomedullin (AM) is a recently identified vasoactive peptide originally discovered from the tissue extract of pheochromocytoma by monitoring the elevation of cAMP in rat platelets [1]. The peptide consists of 52 amino acids and 1 intramolecular disulphide bond and is produced in several tissues, including adrenal medulla, lung, kidney and heart [2].

In addition, it has been demonstrated that endothelial cells synthesize and secrete actively AM [3] and that specific receptors for AM are present in cultured smooth muscle cells [4]. Moreover, AM has been detected in the plasma [2, 5] and other fluids [6] from normal individuals.

When injected intravenously into rats, AM elicits a strong, long-lasting hypotension, as a consequence of vasodilatation in resistance arteries [1]. Recently, AM plasma concentration has been reported to be higher in patients with essential and secondary hypertension [7–9], heart failure [10] and myocardial infarction [11], compared with normal subjects.

These findings indicate that AM may be involved in the regulation of vascular tone and in circulatory control.

Cardiac transplantation has been used with increasing success for the treatment of patients with end-stage congestive heart failure. After cardiac transplantation patients show a high incidence of systemic hypertension [12, 13] and a remarkable finding in patients receiving cyclosporine-A is the frequent development of systemic arterial hypertension [14–16]. This observation is in sharp contrast to that in patients receiving azathioprine and prednisone, in whom post-operative hypertension developed in less than 20 % [17].

Cyclosporine-A can increase blood pressure through many mechanisms: up regulation of angiotensin II receptors and calcium responses in vascular smooth muscle cells [18], inducing a decreased production of NO following, and endothelial dysfunction [19], through an activity on endothelin receptors [20].

The current study was designed: (1) to investigate if AM plasma levels are modified in patients following heart trans-

plantation as compared to healthy subjects and patients with essential hypertension; (2) to determine if a correlation between AM plasma and some haemodynamic parameters, serum creatinine and plasma cyclosporine levels exists.

Methods

We studied 3 groups of subjects: group 1 included 16 consecutive non-smoker patients (10 men and 6 women), who underwent an orthotopic heart transplantation at Institute of Cardiac Surgery of University of Rome "La Sapienza" more than 6 months before, were enrolled in the study. Their mean age was 52.1 ± 9.8 (range 35–64) years and body mass index

Table 1. Characteristics of patients with orthotopic heart transplantation

N°	Age (years)	BMI (kg/m ²)	Heart disease diagnosed	Medications
1	56	25.17	DC	Cys-A; Pred; AZA; FU
2	38	23.97	DC	Cys-A; Pred; AZA
3	60	27.48	DC	Cys-A; Pred; AZA
4	51	24.22	CAD	Cys-A; Pred; FU
5	58	23.88	CAD	Cys-A; Pred
6	47	23.84	CAD	Cys-A; Pred; AZA
7	64	27.98	DC	Cys-A; AZA; FU
8	45	26.51	DC	Cys-A; AZA; Pred
9	39	26.66	DC	Cys-A; Pred; AZA; FU
10	66	23.45	CAD	Cys-A; AZA; FU
11	57	22.65	CAD	Cys-A; AZA; FU
12	60	26.98	CAD	Cys-A; AZA
13	35	21.10	DC	Cys-A; Pred; AZA
14	57	27.33	DC	Cys-A; Pred; AZA
15	59	20.70	DC	Cys-A; Pred; AZA
16	42	22.22	DC	Cys-A; Pred; AZA

BMI = body mass index; DC = primitive dilated cardiomyopathy; CAD = coronary artery disease; Pred = prednisone; AZA = azathioprine; FU = furosemide; Cys-A = Cyclosporine A.

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(BMI) was 24.6 ± 2.3 . In all patients the last endomyocardial biopsy was negative for myocardial rejection. Patient characteristics at entry are listed in Table 1.

Only three patients were known to have mild hypertension before cardiac transplantation. All patients had good left ventricular function with an ejection fraction above 50 %, as determined by echocardiography performed the same day of blood sampling for the present study.

All patients were in stable clinical condition without signs and symptoms of heart failure and were not hospitalized.

Group 2 included 21 normotensive healthy subjects (15 men and 6 women, mean age 31 ± 12 years) recruited from volunteers employed in our hospital.

In Group 3 were enrolled 15 patients with essential hypertension (10 men and 5 women, mean age 42 ± 15 years).

Subjects and patients were excluded from the study if any following criteria were found: heart failure, pregnancy and pre-eclampsia, diabetes, liver disease, hyperthyroidism, Addison's disease, sepsis, acute asthma and chronic renal failure.

No subjects and patients were overweight (BMI < 27) and none of them was a smoker.

All essential hypertensive patients were in stage I (without signs of organ damage) according to the World Health Organization. Blood pressure was measured on at least three consecutive occasions, 15 minutes after the patient assumed the supine position.

Reported values were mean of at least three different evaluations. Systemic hypertension was defined as systolic blood pressure > 160 mmHg, diastolic blood pressure > 95 mmHg, or both. All drugs, except immunosuppressive agents (cyclosporine-A, prednisone and azathioprine) and diuretics (if required), were discontinued 2 weeks before the study. All patients were allowed to continue their daily diet with the usual intake of sodium, potassium and proteins.

The day of the study subjects and patients were kept in the supine position for at least 30 minutes. Blood pressure was measured four times at three minute intervals by means of a standard Riva-Rocci manometer with a cuff of appropriate size and a stethoscope located at the brachial artery was used. The first measurement was discarded and the mean of the last three pressures was calculated.

10 ml of venous blood was collected from the antecubital vein between 08.00 and 09.00 a. m. after overnight fasting. Five ml of blood were collected in polystyrene tubes containing EDTA (1 mg/ml) and aprotinin (500 KIU/ml). Blood samples were then centrifuged at $3000 \times g$ at 0 °C for 15 minutes. The plasma and serum were immediately frozen and stored in glass tubes at -70 °C and -20 °C, respectively, until assayed.

AM plasma concentrations were measured by specific radioimmunoassay (RIA) (Phoenix Pharmaceuticals, Inc., Mountain View, CA, USA) after extraction and purification. Briefly, 2 ml of plasma was applied to conditioned Sep-Pak C-18 cartridge (Millipore Corp., Waters Chromatography, Milford, MA, USA) and the column was sequentially washed with

5 ml of isotonic saline, 5 ml of 0.1 % trifluoroacetic acid and 5 ml of 20 % acetonitrile in 0.1 % trifluoroacetic acid. Then, the absorbed material was eluted with 4 ml of 50 % acetonitrile and the eluate was lyophilized. The residue was dissolved in 0.3 ml of 50 mMol phosphate buffer (pH 7.4) and was submitted to RIA using the radioiodinated AM and antiserum against synthetic AM in rabbits. According to the manufacturer's instructions, the antibody crossreacts 100 % with human AM (1-52) but not with rat AM (1-50), human amylin, human CGRP, endothelin-1, α -ANP-(1,28), β -ANP-(32) or ACTH.

The effective range of the standard curve was between 2 and 200 pg of human AM per assay tube. The interassay variation was 12 % and intraassay variation was 5 %. All assays were performed in duplicate. Concentrations of AM were expressed as pg/ml. Serum creatinine, sodium and potassium were measured by routine laboratory methods. Cyclosporine measurements were made on a TDX monoclonal system (ABBOT).

All data are given as mean $\pm$ standard deviation. The statistical calculation was performed using "Primer" software (Primer of Biostatistics, S. A. Glantz, Mc Graw-Hill, S. Francisco, 1987). The individual values were inserted by group on the spread sheet and were evaluated by one-way ANOVA and Bonferroni's t-test, whenever appropriate. Correlations between AM values and other variables were determined by means of linear regression analysis. A p value < 0.05 was considered statistically significant.

Results

Eleven out of 16 (68.7 %) patients after cardiac transplantation were hypertensives (systolic pressure > 160 mmHg, diastolic pressure > 95 mmHg) (Table 2). In these patients serum creatinine was greater in respect to normotensive heart transplanted patients ($p < 0.05$) and controls ($p < 0.05$).

Plasma cyclosporine levels were not different between normotensive and hypertensive transplanted patients (Table 2).

In group 1, AM plasma level was 23.7 ± 8.6 pg/ml. This value was statistically different ($p < 0.05$) from that measured in 21 normal subjects (13.7 ± 6.1 pg/ml, Figure 1). In this series of cardiac transplant recipients, AM plasma levels were statistically different ($p < 0.05$) between hypertensive (25.5 ± 9.7 pg/ml) and normotensive (20.5 ± 4.4 pg/ml) patients. AM plasma levels did not correlate with blood pressure, heart rate, plasma cyclosporine concentrations, or serum creatinine in either group.

AM plasma levels in group 3 were higher (22.7 ± 8.2 pg/ml) with respect to normal subjects (13.7 ± 6.1 pg/ml; $p < 0.05$) but not statistically different from values measured in group 1 (23.7 ± 8.6 pg/ml, $p > 0.05$; Figure 1). In particular, no difference was found between AM plasma values in essential hypertensive patients (22.7 ± 8.2 pg/ml) and that in transplanted hypertensive patients (25.5 ± 9.7 pg/ml, $p > 0.05$).

Table 2. Blood pressure, heart rate, serum electrolytes, serum creatinine and plasma cyclosporin-A in all groups

Variables	Heart transplant patients		Essential hypertensive patients (n = 15)	Healthy subjects (n = 21)
	Hypertensive (n = 11)	Normotensive (n = 5)		
Systolic blood pressure (mmHg)	$160.4 \pm 4.15^*$	129.1 ± 2.1	$158.4 \pm 4.21^*$	119.1 ± 6.1
Diastolic blood pressure (mmHg)	$101 \pm 4.5^*$	84.1 ± 5.1	$102.3 \pm 3.8^*$	76.2 ± 6.1
Heart rate (b/min)	$77 \pm 11^{**}$	$80 \pm 15^{**}$	70 ± 2	68 ± 4
Na ⁺ (mEq/l)	141.8 ± 4.2	141.7 ± 5.4	139.5 ± 2.3	139.1 ± 2.1
K ⁺ (mEq/l)	4.4 ± 0.5	$4.5 \pm 0.3^*$	4.1 ± 0.3	3.9 ± 0.4
Serum creatinine (mg/ml)	$1.58 \pm 0.4^*$	1.1 ± 0.1	0.8 ± 0.3	0.8 ± 0.2
Cyclosporine-A (ng/ml)	180.3 ± 65.7	169.8 ± 54.5		

*p < 0.05 vs healthy subjects and normotensive heart transplant patients; **p < 0.05 vs healthy subjects

Discussion

The data from our study showed that AM plasma concentrations were significantly higher in heart transplant recipients as compared to healthy subjects. These results confirm and extend the data recently reported by Geny and co-workers [21] who observed high values of plasma AM in nine heart-transplant recipients, early and late after transplantation. In addition, in our patients, AM plasma concentrations were higher in hypertensive heart transplant recipients with respect to normotensives. Furthermore, we showed that AM plasma levels were significantly higher in patients with essential hypertension if compared to normal subjects.

However, hypertensive patients and transplanted patients had similar AM plasma levels.

These data regarding AM plasma level in essential hypertension are confirmatory [7]. Other studies [22] speculated that the increase in AM plasma concentration in essential hypertensive patients is determined by high blood pressure.

The mechanism of the increase in this peptide following cardiac transplantation remains unknown.

At present, the synthesis, secretion and metabolism of AM are not fully understood. AM was shown to be present in high concentrations in the adrenal medulla and was also detected in various organs, such as heart (atrium and ventricle), kidney, lung and vascular endothelium [1–4].

Nishikimi and co-workers [5] have recently investigated the sites of production and clearance of AM in humans. The results of their study suggest that adrenal glands are not the main source of circulating AM in respect to other organs and that the lungs may be one site of AM clearance.

AM appears to be actively synthesized and secreted by vascular endothelium into both the bloodstream and space between endothelial cells and vascular smooth muscle cells [3, 4]. Thus it is likely that vascular tissues are one of the main sources of circulating AM.

Although there is no difference between plasma AM levels in all heart transplanted patients compared to patients with essential hypertension it is not possible to ascertain if the levels of AM do reflect a response to hypertension or other mechanisms can be involved. An abnormal endothelial func-

tion seems to occur after heart transplantation and the mechanism for this dysfunction could be related to cyclosporine treatment. In fact, some authors have found that the exposure to cyclosporine of bovine aortic endothelial cells in culture is associated with vascular damage [23].

Therefore, we can hypothesize that the increase of AM plasma concentrations, found in our heart-transplant recipients, can be determined by the endothelial cell injury induced by cyclosporine treatment. However, this hypothesis is not fully supported by the results of Geny et al. [24] that have demonstrated that clinically relevant cyclosporine-A therapy does not acutely increase AM plasma levels in six heart transplant recipients.

After cardiac transplantation, cyclosporine-treated patients have a high incidence of systemic hypertension [14–16]. This post-transplant hypertension is characterized by elevated systemic vascular resistances, normal cardiac output, and a mild impairment of renal function [25, 26].

In the present study AM plasma concentration was significantly higher in hypertensive patients following cardiac transplantation with increased serum creatinine (> 1.2 mg/dl) as compared with normotensive heart transplant recipients and normal creatinine.

Other investigators reported that AM plasma concentration is increased in patients with renal failure [20, 27], in hypertensive patients with increased creatinine (≥ 1.2 mg/dl) [20] and in congestive heart failure patients with and without increased creatinine (≥ 1.2 mg/dl) [10, 28].

These findings suggest that elevation of AM plasma concentration may be in part due to the reduction in renal function.

In conclusion, the present study shows that AM plasma levels were higher in all cardiac transplant recipients compared to healthy subjects. Moreover, in cardiac transplant recipients plasma AM concentrations were higher in hypertensive with respect to normotensive patients. Our data suggest that this peptide may be, partly, involved in the vascular tone and in circulatory control after heart transplantation.

The present study does not exclude the possibility that increased plasma AM after cardiac transplantation is related to elevated pre-transplant levels.

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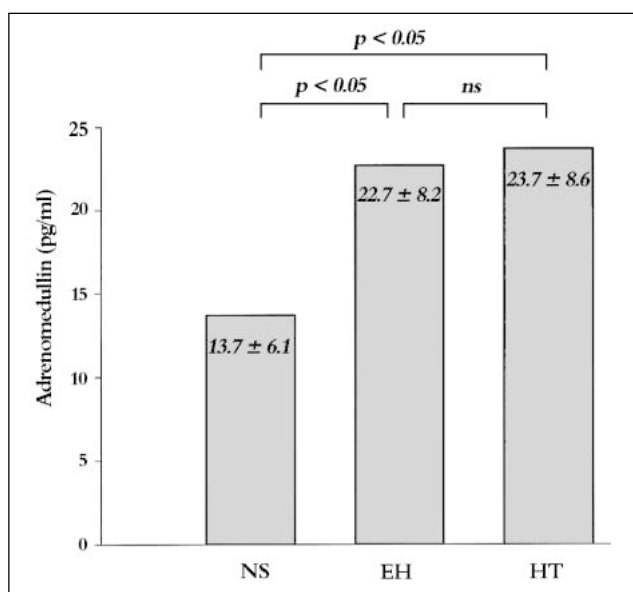


Figure 1. Mean plasma adrenomedullin values in all groups (NS = normal subjects; EH essential hypertensive patients; HT = heart transplanted patients)

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