Circulating adrenomedullin is increased after heart transplantation

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Abstract

**Objective:** Adrenomedullin (ADM), secreted by the failing human heart, is a newly discovered potent endogenous vasorelaxing and natriuretic peptide that may play a role in cardiorenal regulation. No data are available on ADM in heart-transplant recipients (Htx) and the aim of this study was to determine the short- and long-term responses of ADM after heart transplantation. **Methods:** Circulating ADM and its relationship with parameters of cardiovascular hemodynamics, humoral factors and renal function were determined in normal subjects and Htx early (1, 2, 4, 8, 15 and 30 days) and late (32±16 months) after transplantation. Additionally, ADM was obtained in matched hypertensive and renal-transplant patients (n=9 in each group). **Results:** Plasma ADM, elevated in heart failure patients, further increased transiently at day 1 after transplantation (from 37.9±15.9 to 125.8±15.3 pmol/l, P<0.01) and, although decreasing thereafter, remained elevated until the 30th day after transplantation (52.1±25.2 pmol/l). Late after transplantation, ADM concentrations were still increased compared to normal values (31.3±5.3 vs. 19.4±2.7 pmol/l, P<0.001). ADM positively correlated with endothelin, atrial natriuretic peptide (ANP) and cyclosporine. ADM was also correlated with increased diastolic (r=0.68, P<0.04) and systolic (r=0.66, P<0.05) blood pressure in late Htx. No relationship was observed between ADM and left ventricular mass index, aldosterone and creatinine. ADM elevation was similar in hypertensive, renal-transplant patients and in Htx. **Conclusions:** Circulating ADM is increased after heart transplantation, in relation to hypertension, endothelin, cyclosporine and ANP. In view of ADM’s biological properties, these results might suggest a compensatory role for ADM against further development of vasoconstriction and fluid retention states after heart transplantation. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Adrenomedullin; Human-heart transplantation; Atrial natriuretic factor

1. Introduction

Originally isolated from human pheochromocytoma, adrenomedullin (ADM) is a newly discovered potent endogenous vasorelaxing and natriuretic peptide [1–6]. Present mainly in adrenal medulla, in the cardiovascular system and in kidneys, ADM is produced by vascular smooth muscle cells and by vascular endothelial cells and has been recently reported to be secreted by the failing human heart [7]. ADM is observed in normal human plasma [8] and its concentration increases in patients with hypertension, chronic renal failure [9,10], and in congestive heart failure in proportion to the severity of the disease [7,11,12]. These recent studies suggest that ADM may oppose the co-activation of local and circulating vasoconstrictive and sodium-retaining factors during heart disease. Heart transplantation is an important surgical treatment for end-stage congestive heart failure, replacing cardiac systolic function and normalizing partially the neuro-humoral activation. Thus, plasma catecholamines, arginine vasopressin and renin–angiotensin–aldosterone values normalize in heart-transplant recipients (Htx). On the other hand, endothelin together with atrial and brain natriuretic peptides plasma levels have been generally reported to be elevated in Htx [13–17]. To date, despite its potential beneficial properties, no data has been reported on ADM after heart transplantation.

This study was therefore designed to determine the circulating ADM levels in heart-transplant recipients, early...
and late after transplantation, and to investigate ADM’s clinical significance through correlations between its plasma levels and variables of cardiovascular hemodynamics, renal function and hormonal factors implicated in blood pressure and body-fluid regulation.

2. Methods

The study was approved by the Institutional Review Board for Human Studies and all subjects gave informed consent.

2.1. Study design

Venous blood samples were obtained in all subjects after a 30 min rest period in the supine position. Blood was drawn in normal subjects and in Htx early (1, 2, 4, 8, 15 and 30 days) and late (32±16 months) after transplantation. Other body fluid regulatory hormones, such as atrial natriuretic peptide (ANP), endothelin and aldosterone, together with plasma creatinine, which is used as an index of renal function, and cyclosporine levels were also determined simultaneously in Htx. To assess the effect of systemic hypertension and of cyclosporine therapy, circulating ADM together with plasma creatinine and the cyclosporine levels were additionally determined in matched hypertensive subjects and renal-transplant recipients (Rtx).

2.2. Study population

2.2.1. Early and late heart-transplant recipient group

Nine patients, who needed heart transplantation for end-stage congestive heart failure, were studied from just before to 30 days after transplantation. They usually received chronotropic and inotropic supports such as isoproterenol and/or dobutamine, dopamine and catecholamines during the first week after transplantation. Immunosuppressive therapy was induced with equine antilymphocyte globulin (Mérieux, France) during the first five postoperative days in association with a 2 mg/kg/day dose of prednisone that was rapidly decreased to 1 mg/kg/day at day 3. Azathioprine was prescribed at an initial dose of 2–3 mg/kg/day, adjusted to keep the white blood cell count >4000. Cyclosporine was introduced on the fourth postoperative day, at an initial dose of 3 mg/kg, titrated to maintain plasma levels at between 210 to 290 nmol/l. All but five of the 25 endomyocardial biopsies performed during the time of the study were free of rejection. The four 1A and the only 3A grade rejection episodes were rapidly resolved, as shown by the following biopsy. To avoid the unstable period just following surgery and, except in Fig. 1 showing the plasma ADM time course, the data presented in the tables concerning the early Htx group are the means of all values obtained from the eighth to the 30th day after transplantation.

Nine late Htx, age- and weight-matched, were studied 32±16 months after transplantation. All, except one showing a grade 1A rejection, were free of rejection, as shown by the nearest endomyocardial biopsy, which were performed 46±41 days apart from the study. Htx received the usual triple immunosuppressive therapy with prednisolone (10.3±3.3 mg/day), cyclosporine with a whole blood trough level at 116±48 nmol/l and azathioprine (43.3±28.2 mg/day). Htx also received antihypertensive treatment either by nitrates (n=1), calcium antagonists (n=2), angiotensin conversion enzyme inhibitors (n=1) and/or furosemide (n=3).

2.2.2. Normal subjects, hypertensive and renal-transplant recipients

Nine matched normal subjects, with no history or symptoms of cardiovascular disease, comprised the normal group (controls). The hypertensive (HTA) and renal-transplant (Rtx) patient groups were additionally investigated in order to determine a possible effect of hypertension, related or not to cyclosporine therapy, on ADM plasma values. These patients were matched with the late Htx, considering age, weight, systemic blood pressure, creatinine and cyclosporinemia (Table 1). The antihypertensive treatment of the hypertensive patients was composed either of beta-blockers (n=5), nitrates (n=2), calcium antagonists (n=3), angiotensin conversion enzyme inhibitors (n=2), central antihypertensive therapy (n=2) and/or diuretics (n=2). In renal-transplant patients, the hypertensive therapy comprised beta-blockers (n=2), nitrates (n=2), calcium antagonists (n=4) and/or diuretics (n=2).

2.3. Radioimmunoassays

Blood for hormone assays was immediately placed on ice in EDTA–aprotinin tubes to prevent proteolytic break-
ventricular posterior wall thickness (PWT) were similar in late Htx and in Rtx. Inter-
ventricular septum thickness (IVST) and left decreased over time after heart transplantation and were
pared to controls. Finally, cyclosporine trough levels
determined by an immunoenzyme assay using the kit from
sur Yvette, France). Total blood cyclosporinemia was assessed with one-way ANOV A. When ANOV A was
was 0.8 pmol / l. Plasma aldosterone was determined by
radioimmunoassay using kits from Amersham (Buckin-
ghamshire, UK) after extraction by Sep.Pak C cartridges
Waters). The sensitivity of the assay was 0.6 pmol / l. The intra-assay coef®cient of variation was 7%. Other
hormone measurements were made as previously reported [14,15]. Briefly, plasma ANP was determined by
radioimmunoassay using kits from Amersham (Buckin-
ghamshire, UK) after extraction by Sep.Pak C cartridges
Waters). The sensitivity of the assay was 2 pmol / l. All of the results are expressed as means
m 6
L VM index (g / m 2)
96±24
108±38f
2.5. Echocardiography
Echographic data were obtained, in normal subjects and Htx, after 10 min of rest with the subject in the left
decubitus position, using an Advanced Technology Lab-
atories Ultramark 9 echodoppler and a 2.25 MHz
transducer. Left ventricular end-diastolic dimension
(LVD), interventricular septum thickness (IVST) and left ventricular posterior wall thickness (PWT) were deter-
mined using the left parasternal long axis view, according
to the recommendations of the American Society of
Echocardiography. Left ventricular mass (LVM) was then
calculated from the Penn convention, according to the
equation of Devereux and Reichek: LVM = 1.04[(IVST+
PWT+LVD) 3−LVD 3]−13.6. The left ventricular mass
index (LVMi) was calculated by dividing the left ventricu-
lar mass by the body surface area.

2.6. Statistical analysis
All of the results are expressed as means±SD. Differ-
ences in the time course of plasma ADM after transplanta-
tion were assessed using a two-way ANOVA (effect of
transplantation and effect of days after surgery) with
repeated measures. Other differences between groups were
assessed with one-way ANOVA. When ANOVA was
significant, comparisons between individual means were
performed using the a posteriori Tukey’s test. The relation-
ship between two variables was examined by regression
analysis. The results were considered to be signi®cant at a
level of P<0.05.

3. Results
Table 1 presents the clinical and biological characte-
istics of the normal subjects, the renal-transplant and
hypertensive patients and of the early and late Htx. Age and
body-weight were similar in all groups. Heart rate was
higher in early Htx than in normal and hypertensive
subjects. Systemic blood pressure was similarly increased
in hypertensive patients, in Rtx and late Htx compared
to controls. The left ventricular mass index of Htx was not
significantly different from that of normal subjects. Plasma
creatinine was similarly increased in Rtx and Htx, com-
pared to controls. Finally, cyclosporine trough levels
decreased over time after heart transplantation and were
similar in late Htx and in Rtx.

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**Table 1**
Clinical and biological characteristics of the experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Rtx</th>
<th>HTA</th>
<th>Early Htx</th>
<th>Late Htx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46±10</td>
<td>45±15</td>
<td>58±9</td>
<td>55±10</td>
<td>46±11</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>79±12</td>
<td>70±11</td>
<td>86±14</td>
<td>75±8</td>
<td>76±16</td>
</tr>
<tr>
<td>Delay (months)</td>
<td>−</td>
<td>66±39</td>
<td>−</td>
<td>0.3–1</td>
<td>32±16f</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>72±9</td>
<td>79±14</td>
<td>64±11f</td>
<td>92±17f,4</td>
<td>83±12f</td>
</tr>
<tr>
<td>Systolic SBP (mmHg)</td>
<td>120±14</td>
<td>142±12f</td>
<td>139±8f,5</td>
<td>126±8</td>
<td>138±11f</td>
</tr>
<tr>
<td>Diastolic SBP (mmHg)</td>
<td>66±13</td>
<td>79±13</td>
<td>79±3f</td>
<td>73±5</td>
<td>79±10</td>
</tr>
<tr>
<td>Mean SBP (mmHg)</td>
<td>84±14</td>
<td>100±12f</td>
<td>99±4f</td>
<td>91±5</td>
<td>99±9f</td>
</tr>
<tr>
<td>LVM index (g/m²)</td>
<td>96±24</td>
<td>−</td>
<td>−</td>
<td>110±13</td>
<td>103±15</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>90±12</td>
<td>136±36f</td>
<td>96±23</td>
<td>133±28f</td>
<td>116±14f</td>
</tr>
<tr>
<td>Cyclosporine (nmol/l)</td>
<td>−</td>
<td>108±38f</td>
<td>−</td>
<td>490±250</td>
<td>116±46f</td>
</tr>
</tbody>
</table>

Values are expressed as means±SD. Rtx, renal-transplant patients; HTA, hypertensive patients; Htx, heart-transplant patients; SBP, systemic blood
pressure; LVM, left ventricular mass.
Differences between means (ANOVA and Tukey’s test): difference with controls: *P<0.05, **P<0.01; difference with HTA: †P<0.05 and ‡P<0.01;
difference with early Htx: ’P<0.05, ’’P<0.01; difference with Rtx: ′P<0.05.
A plasma ADM time course, early after heart transplantation, is presented on Fig. 1, together with the range of normal values. Thus, ADM was elevated in patients with heart failure just before transplantation (day 0), compared to controls (37.9 ± 15.9 vs. 19.4 ± 2.7 pmol/l, P < 0.05). Heart transplantation induced a transient ADM increase at day 1 (from 37.9 ± 15.9 to 125.8 ± 15.3 pmol/l, P < 0.01) and, although decreasing progressively thereafter, ADM remained elevated until the 30th day after transplantation (52.1 ± 25.2 pmol/l), compared to the control values.

Plasma ADM values in the five groups of subjects are shown in Fig. 2. ADM is increased in renal-transplant, hypertensive and early and late heart-transplant patients, compared to controls (44.7 ± 5.3 pmol/l in Rtx, 55.4 ± 16.5 pmol/l in HTA, 49.9 ± 14.9 pmol/l in early and 31.3 ± 5.3 pmol/l in late Htx). Circulating ADM levels in our normal subjects were also increased, compared to previously reported values [1,7,12]. However, the approximately twofold rise in the mean plasma ADM levels in hypertension and heart failure patients observed in our study was similar to the change in ADM reported by other groups [10–12,18], although the absolute values differed.

The plasma levels of body-fluid and blood pressure regulatory hormones, determined in controls and in early and late Htx, are presented on Table 2. Plasma ANP was significantly increased both in early and late Htx. Endothelin and aldosterone, increased early after transplantation, decreased significantly thereafter, so that they did not differ significantly from controls late after heart transplantation.

To investigate the clinical significance of increased ADM in Htx, we determined the correlations between ADM and the hormonal, biological and hemodynamic characteristics of the patients early and late after heart transplantation. The results are presented in Table 3. ADM positively correlated with endothelin (r = 0.51, P < 0.004 and r = 0.86, P < 0.003, early and late, respectively, after heart transplantation). A positive correlation was also observed between ADM and ANP early (r = 0.54, P < 0.008) but it just failed to reach statistical significance late after transplantation (r = 0.62, P = 0.07). ADM and systemic blood pressure were positively correlated late after heart transplantation (r = 0.68, P < 0.04 and r = 0.66, P < 0.05 for diastolic and systolic blood pressure, respectively) and with heart rate early after transplantation (r = 0.59, P < 0.005). Finally, ADM and cyclosporine were correlated both early and late after transplantation (r = 0.41, P < 0.007 and r = 0.66, P < 0.05, respectively). No relationship was observed between ADM and the left ventricular mass index, aldosterone or creatinine. A positive correlation between endothelin and cyclosporine (r = 0.59, P < 0.001) was also observed in Htx. No significant correlation was observed between ADM and blood pressures or creatinine.
in HTA and Rtx and between ADM and cyclosporine in Rtx.

4. Discussion

The present study demonstrates, for the first time, that circulating ADM is increased, early and late, after heart transplantation and that ADM positively correlates with blood pressure, endothelin, cyclosporine and ANP in Htx. Interestingly, ADM is also similarly elevated in matched hypertensive and renal-transplant patients. The origin of increased circulating ADM after heart transplantation is unknown, but it may result from decreased clearance and/or increased production. Few data are available concerning the clearance pathway of ADM, but if the adrenal glands and the heart are not important catabolism sites, ADM appears to be degraded in human lungs [19,20]. In patients with chronic renal failure, plasma ADM levels are positively correlated with serum creatinine and inversely correlated with the glomerular filtration rate, in proportion to the severity of renal impairment [9,10]. Consequently, decreased ADM renal clearance may participate in ADM elevation in Htx. However, consistent with data reported in heart failure patients [7], such a mechanism is unlikely to play a key role after cardiac transplantation in view of the very mild degree of renal impairment and the lack of correlation between ADM and serum creatinine observed in our Htx.

Direct ventricular secretion of ADM has been reported in patients with heart failure [12] and plasma ADM level has been related to cardiac hypertrophy in animals [21]. Accordingly, plasma ADM elevation in patients with untreated essential hypertension was recently proposed to be partly related to cardiac hypertrophy [22]. Despite the lack of relationship between ADM and the LVM index, which might be explained by the relatively small increase in Htx’s cardiac thickness, hypertension is thus likely to participate in ADM release after heart transplantation. Indeed, a positive correlation was observed in late Htx between ADM and systemic blood pressure, and ADM was similarly increased in matched hypertensive patients and renal-transplant recipients.

Interestingly, although these data support a role for hypertension per se in ADM release, whether or not related to cyclosporine therapy, Htx are characterized by their specific relationship between ADM and blood pressure, endothelin and cyclosporine. Endothelin is a powerful stimulus for ADM production by vascular cells [23] and the positive correlation observed between ADM and endothelin after heart transplantation supports the view that endothelin might participate in the ADM increase in Htx. Indeed, although a correlation cannot establish a causal relationship between two parameters, the significant and important relationship observed between endothelin and ADM suggests that more than 60% of the plasma ADM variations may be related to plasma endothelin variations late after heart transplantation. In this regard, the positive correlations between cyclosporine and ADM, on the one hand, and between cyclosporine and endothelin, on the other, might suggest that cyclosporine partly increases ADM secretion in Htx through its stimulating action on endothelin secretion [24].

Besides endothelin, multiple local and circulating factors, such as cytokines, ANP and steroids, have been shown to increase ADM production from vascular endothelial and smooth muscle cells [3–5]. Thus, as in other cardiac surgery procedures, cardiopulmonary bypass-induced cytokine release may explain the transient ADM increase observed at day 1 after heart transplantation [4]. Indeed, a positive correlation between ADM increment and aortic cross-clamp time was observed in coronary-artery-bypass patients [25,26]. ANP may also participate in ADM elevation in Htx since it has been shown that ANP infusion increases circulating ADM in healthy humans [27]. Alternatively, as in heart failure patients [11], the correlation between ANP and ADM may suggest that ADM may be stimulated by increased body fluid volume after heart transplantation [10–12,28]. Finally, the dose-dependency of the dexamethasone-induced up-regulation of the gene encoding for ADM may explain why low maintenance steroids therapy did not appear to stimulate ADM secretion in Htx [29,30].

Whether or not increased ADM in Htx may serve as a pathophysiological marker or play a biological role remains to be determined. As recently demonstrated, circulating ADM cannot discriminate between mild congestive heart failure patients and healthy subjects [31] but, in view of its potent and long-lasting natriuretic and vasodilatory effects [5] and of its paracrine actions, increased ADM in Htx may have a clinical significance. One may thus speculate that, as in heart failure patients [11], ADM may be involved in the defense mechanisms against further volume expansion and against further systemic and/or local vascular resistance increases. Indeed, ADM has been shown to increase local blood flow in heart and kidneys [32,33] and ADM may thus counteract an endothelin-induced renal vasoconstriction and/or heart vasculopathy in Htx [34]. Consistent with this theory, human ADM has been shown to inhibit the endothelin production of vascular smooth muscle cells [35], suggesting that the correlation between ADM and cyclosporine may correspond to a protective effect of ADM against cyclosporine-induced vasoconstriction, which has been shown to be partly mediated by endothelin production enhancement [24].

In conclusion, circulating ADM is increased, early and late, after heart transplantation. This increase, similar to that observed in matched hypertensive patients, whether or not in need of immunosuppressive therapy, supports a role for hypertension per se in ADM release in Htx. However, the positive correlations observed specifically in Htx between ADM and blood pressure, endothelin, cyclospor-
rine and ANP also suggest that increased ADM might be regarded as a compensatory mechanism to offset further development of cardiac or renal dysfunction, which is probably related to endothelin and/or cyclosporine therapy after heart transplantation. Further studies will be needed to investigate such an hypothesis.

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References