Plasma levels of mature form of adrenomedullin in patients with haemodialysis

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Abstract

Background. Adrenomedullin (AM) is a potent vasodilator and natriuretic peptide with hypotensive effects. Immunoreactive AM in human plasma consists of the biologically active mature form, AM (1-52)-CONH₂ (mAM) and the intermediate form, AM-gly-COOH (iAM). However, the different effects of mAM and iAM in patients on haemodialysis (HD) have remained unclear.

Methods. Thirty-nine patients on HD and 10 controls were included in this study. We determined plasma levels of mAM and iAM using an immunoradiometric assay that recognizes total AM (tAM) and another that is specific for only mAM.

Results. The plasma concentrations of mAM and iAM in patients before HD were significantly higher than those in the controls (n=10) (4.76 ± 0.28 vs 1.28 ± 0.22 fmol/ml, P < 0.001, 25.99 ± 1.47 vs 8.52 ± 0.91 fmol/ml, P < 0.001 respectively). The plasma levels of mAM and iAM before HD significantly and negatively correlated with systolic blood pressure (SBP) (r = -0.46, P < 0.01, and r = -0.32, P < 0.05 respectively) and diastolic blood pressure (DBP) (r = -0.32, P < 0.05, and r = -0.35, P < 0.05 respectively). After HD, plasma mAM and iAM levels as well as SBP and DBP were significantly lower than before HD. Plasma levels of mAM and iAM correlated significantly (r=0.73, P < 0.001).

Conclusions. These data suggest that mAM and/or iAM are involved in blood pressure regulation in patients undergoing HD, and further work is needed to understand the precise role of adrenomedullin in this regulation.

Keywords: adrenomedullin; haemodialysis; hypertension; intermediate form; mature form

Introduction

Adrenomedullin (AM) is a potent vasodilator peptide that was discovered in human phaeochromocytoma by monitoring increases in platelet cyclic adenosine monophosphate (AMP) activity [1]. An intravenous injection of AM into dogs and rats increased the glomerular filtration rate and fractional sodium excretion, indicating that AM acts as a natriuretic factor [2-4]. Human AM is a 52 amino acid peptide with an amidated C-terminal tyrosine and a ring structure formed by a disulphide bridge between adjacent cysteine residues [5]. Adrenomedullin may be synthesized as glycine-extended AM (iAM), which is a biologically inactive intermediate, and the C-terminus is then enzymatically amidated for conversion into the biologically active form, hereinafter called mature AM (mAM) [5,6]. Recent studies have shown that levels of circulating AM are significantly increased in patients undergoing haemodialysis (HD) [7,8]. However, plasma molecular forms of AM have not been examined to date. An immunoradiometric assay (IRMA) for human mAM using monoclonal antibodies that are highly specific for the ring structure and the amidated C-terminal structure of AM has been developed [9]. We used this assay to examine plasma mAM levels in patients undergoing HD. We also determined the total AM (tAM) level in the same individuals, which enabled us to calculate the iAM concentration by subtracting the amount of mAM from that of tAM [6,10].

Subjects and methods

Patients and sampling

Thirty-nine patients undergoing HD aged 29–80 years (mean age, 61.8 ± 2.0 years (SEM); 18 men and 21 women) and 10 healthy individuals aged 25–35 years (mean age: 28.9 ± 1.1 years, seven men and three women) participated in the present study (Table 1). Patients with congestive heart failure were excluded. The underlying renal diseases of the
HD patients were as follows: chronic glomerulonephritis (n = 17), diabetes mellitus (n = 8), polycystic kidney disease (n = 3), nephrosclerosis (n = 3), gout (n = 2), and other (n = 6). Nine of the patients were not taking anti-hypertensive drugs; the other 30 were on anti-hypertensive medications, including calcium-channel antagonists (n = 21), inhibitors of the renin–angiotensin system (n = 13), beta-adrenergic antagonists (n = 2), and alpha-adrenergic antagonists (n = 2). Some patients were using two or three of these medications at the same time.

Twenty-one patients were on HD using synthetic (high-flux) membranes and 18 patients on HD used cuprophane (low-flux) membranes.

We divided the patients into three subgroups according to their pre-HD mean blood pressure (MBP) values. Group 1 (G 1) consisted of 11 patients with a value of < 100 mmHg; group 2 (G 2) consisted of 20 with a value of ≤ 100 to < 120 mmHg; group 3 (G 3) consisted of eight patients with a value of ≥ 120 mmHg.

The dialysate was buffered with bicarbonate containing 142 mmol/l of sodium. The blood flow was maintained at a rate of 200 ml/min and the dialysate flowed at a rate of 500 ml/min. The cardiothoracic ratio (CTR) was determined from chest X-rays by one examiner. Weight gain was calculated by subtracting the last post-dialysis body weight from that before the start of HD. All the patients gave informed written consent prior to participation in this study.

Blood samples from normal controls were drawn from the antecubital vein early in the morning after an overnight fast, and from the HD patients in the supine position just before starting HD and during the last 5 min of HD. We examined the plasma concentrations of mAM and tAM in 10 patients 30 min after the start of HD. High- and low-flux membranes were used for six and four patients respectively.

Blood was drawn into chilled polypropylene tubes containing EDTA·2Na (1 mg/ml of blood) and aprotinin (500 units/ml of blood). Plasma was obtained by immediate centrifugation at 3000 r.p.m. for 10 min at 4°C. The supernatants were evaluated using IRMAs.

### Assay procedures

Plasma concentrations of mAM and tAM were measured using the mature AM radioimmunoassay (RIA) SHIONOGI and AM RIA SHIONOGI (Shionogi Co., Osaka, Japan) [9]. The assay for mAM does not cross-react with iAM, or with fragments such as AM (12-25), AM (30-41), AM (38-52), AM (46-52) and AM (46-52) NH2. The assay also does not cross-react with proadrenomedullin N-terminal 20 peptide (PAMP), calcitonin gene-related peptide (CGRP), calcitonin, amylin, or neuropeptide Y (NPY). The assay for tAM does not cross-react with PAMP, CGRP, calcitonin, amylin, or NPY. After both tAM and mAM concentrations were determined, the iAM concentration was calculated by subtracting the mAM from the tAM concentration [6,10].

### Statistical analysis

Data are expressed as means ± SEM. Post-dialysis AM concentrations were corrected for ultrafiltration. Simple linear regression was used to analyse correlations. Statistical comparisons between two groups were made using Student’s t-test. Differences were considered significant at a P value < 0.05.

### Results

Table 1 shows the clinical and laboratory data obtained from control and from patients before and after HD. Age, systolic blood pressure (SBP), and MBP in the patients were higher, whereas body weight was lower than those of controls. SBP and MBP significantly decreased after HD compared with these values before HD, concomitant with the reduced body weight.

The mean plasma concentrations of tAM, mAM, and iAM before HD (30.75 ± 1.68, 4.76 ± 0.28, and 25.99 ± 1.47 fmol/ml respectively) were significantly higher than those in normal controls, and significantly decreased after HD (Table 1). Correlations were significant between the plasma concentrations of tAM and mAM (r = 0.81, P < 0.001) (Figure 1A), of tAM and iAM (r = 0.99, P < 0.001) (Figure 1B), and of mAM and iAM (r = 0.74, P < 0.001) before HD. The plasma mAM concentration before HD significantly correlated with SBP and DBP (r = −0.47, P < 0.01, r = −0.32, P < 0.05 respectively) (Figure 2A, B). There was no significant correlation between plasma concentrations of mAM and pulse rate, CTR, or weight gain. The plasma iAM concentration before HD correlated significantly with SBP and DBP (r = −0.32, P < 0.05, r = −0.35, P < 0.05 respectively) (Figure 2C, D). There was no significant correlation between plasma concentrations of iAM and pulse rate, CTR, or weight gain.

The mean plasma levels of tAM, mAM, and iAM among the 21 patients who underwent HD with synthetic membranes decreased significantly (30.99 ± 2.78 fmol/ml before HD to 18.20 ± 1.58 fmol/ml after HD, P < 0.001, 4.93 ± 0.41 fmol/ml to 2.65 ± 0.28 fmol/ml, P < 0.001, and 26.06 ± 2.44 fmol/ml to 15.55 ± 1.45 fmol/ml, P < 0.001 respectively), whereas

### Table 1. Clinical characteristics of control subjects and patients with haemodialysis

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>HD patients (n = 39)</th>
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<tr>
<td></td>
<td>Before HD</td>
<td>After HD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.9 ± 1.1</td>
<td>61.8 ± 2.0*a</td>
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<tr>
<td>Body weight (kg)</td>
<td>64.2 ± 4.8</td>
<td>52.8 ± 1.4*a</td>
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<td>71.3 ± 2.1</td>
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<tr>
<td>SBP (mmHg)</td>
<td>121.3 ± 4.8</td>
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<tr>
<td>DBP (mmHg)</td>
<td>76.5 ± 2.9</td>
<td>82.2 ± 1.7</td>
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<tr>
<td>MBP (mmHg)</td>
<td>91.4 ± 3.4</td>
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<tr>
<td>CTR (%)</td>
<td>ND</td>
<td>51.7 ± 0.9</td>
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<td>Creatinine (mg/dl)</td>
<td>ND</td>
<td>9.9 ± 0.4</td>
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<tr>
<td>Haematocrit (%)</td>
<td>ND</td>
<td>29.2 ± 0.6</td>
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<tr>
<td>T/AM (fmol/ml)</td>
<td>9.80 ± 1.02</td>
<td>30.75 ± 1.68*a</td>
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<tr>
<td>mAM (fmol/ml)</td>
<td>1.28 ± 0.22</td>
<td>4.76 ± 0.28*a</td>
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<tr>
<td>iAM (fmol/ml)</td>
<td>8.52 ± 0.91</td>
<td>25.99 ± 1.47*a</td>
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All values are expressed as mean ± SEM.  
*aP < 0.01 vs controls;  
*bP < 0.05 vs before HD, HD, haemodialysis; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; CTR, cardiothoracic ratio; T/AM, total adenomedullin; mAM, mature adenomedullin; iAM, intermediate adenomedullin.
Mature form of adrenomedullin in HD patients

Fig. 1. Correlations between plasma concentrations of (A) tAM and mAM ($r = 0.81, P < 0.001$), and (B) tAM and iAM ($r = 0.99, P < 0.001$) in 39 patients before haemodialysis.

Fig. 2. Correlations of plasma concentrations of mAM with (A) SBP ($r = -0.47, P < 0.01$) and (B) DBP ($r = -0.32, P < 0.05$), and those of iAM with (C) SBP ($r = -0.32, P < 0.05$) and (D) DBP ($r = -0.35, P < 0.05$) in 39 patients before haemodialysis.

those of the 18 patients who underwent HD with cuprophan membranes did not change ($30.47 \pm 1.77$ fmol/ml before HD to $29.81 \pm 1.54$ fmol/ml after HD, $4.56 \pm 0.37$ to $4.07 \pm 0.27$ fmol/ml, and $25.91 \pm 1.51$ to $25.75 \pm 1.38$ fmol/ml respectively). Ultrafiltration volume, weight gain between HD sessions, and BP change during the HD session did not differ between these two groups.

Plasma mAM levels were $5.53 \pm 0.72$ fmol/ml in G1 (with pre-HD MBP < 100 mmHg), $4.65 \pm 0.31$ fmol/ml in G2 (MBP ≤ 100 to < 120 mmHg), and $3.97 \pm 0.42$ fmol/ml in G3 (MBP ≥ 120 mmHg). The plasma AM concentrations tended to increase as MBP decreased, but not significantly.

The plasma levels of mAM in six patients who underwent HD with high-flux membranes were lower at pre-dialyser, than post-dialyser ($3.66 \pm 0.47$ fmol/ml vs $2.03 \pm 0.51$ fmol/ml, $P < 0.05$). The plasma mAM levels of four patients who underwent dialysis with low-flux membranes did not differ significantly pre- and post-dialyser ($6.34 \pm 2.24$ fmol/ml vs $5.31 \pm 2.29$ fmol/ml, NS).

Discussion

Human AM consists of 52 amino acid residues with a C-terminal amidated Tyr$^{52}$ and a ring structure formed by an intramolecular disulphide bond between Cys$^{16}$ and Cys$^{21}$, both of which are indispensable for the biological activity of AM [1,11]. Immunoreactive AM circulating in the human bloodstream consists of mAM and biologically inactive iAM, the C-terminal of which is not amidated [6]. Plasma AM levels are increased in
chronic renal failure [12] and in patients undergoing HD [7,8], but not in chronic glomerulonephritis with normal renal function [13], suggesting that reduced clearance of AM contributes to the increased plasma level. In HD patients, the plasma AM level may be related to plasma volume and/or blood pressure [7,8], but the molecular forms of plasma AM have not been examined. To clarify the pathophysiological roles of mAM and iAM in HD patients, we examined the plasma levels of these molecular forms and examined the relationship between these values and clinical status, using two novel IRMAs.

The plasma concentrations of tAM and mAM, of tAM and iAM and of mAM and iAM correlated significantly (Figure 1), consistent with the conversion of iAM into mAM by the enzymatic amidation of the C-terminus. The reason for the closer correlation between tAM and iAM than between tAM and mAM is probably that iAM accounts for over 80% of plasma tAM, whereas mAM only amounts to 20% of the total. Thus tAM seems to represent iAM rather than mAM. Plasma AM has been measured in HD patients, but the RIAs used in these studies could not distinguish mAM from tAM. In addition to the difference in the bioactivity of these peptides, the clearance sites of mAM and iAM differ [14]. Measurement of mAM should provide more exact information on the behavior of AM in HD patients.

The plasma concentrations of mAM and iAM before HD were significantly and inversely correlated with SBP and DBP. Khan et al. [15] reported that chronically infused adrenomedullin had a hypotensive effect within the physiological level of plasma AM in rats. Considering its potent vasodilator effects, plasma AM may regulate blood pressure in HD patients. More studies are needed to confirm this hypothesis because this inverse correlation has not been found in other diseases such as chronic glomerulonephritis [13] and heart failure [16]. Although the exact role of iAM has not yet been clarified, iAM in plasma may act as a hormone reservoir for mAM, which can be converted into mAM in order to regulate blood pressure.

The plasma levels of tAM, mAM, and iAM decreased in patients treated by HD with synthetic membranes, but not in those treated with cuprophane membranes. Synthetic membranes generally remove low-molecular-weight proteins more effectively than cuprophane membranes [17,18]. The effects of each membrane type in removing plasma AM by must be considered in HD patients to evaluate the role of AM.

In summary, we measured the plasma levels of mAM and iAM in patients undergoing HD. The mAM and iAM concentrations were significantly higher than those in the control individuals and iAM accounted for over 80% of the total AM. The plasma concentrations of tAM and mAM, of tAM and iAM, and of mAM and iAM correlated significantly. Correlations were inversely significant between the plasma concentrations of AM and blood pressure values before HD, suggesting that mAM plays a role in the regulation of blood pressure in HD patients.

References