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Effects of Hemodialysis on Circulating Adrenomedullin Concentrations in Patients with End-Stage Renal Disease

Abstract

To characterize the determinants of circulating levels of adrenomedullin (AM), the plasma levels of this peptide were measured in 58 patients with end-stage renal disease on hemodialysis. Predialysis plasma levels of AM were more than twice as high in patients on hemodialysis as compared to controls. In hemodialysis patients with heart failure (NYHA classes II–IV) or hypertensive HD patients plasma levels of AM were significantly higher than in patients with end-stage renal disease only. Plasma levels of AM were not altered immediately by hemodialysis but decreased significantly 14–20 h after hemodialysis. AM plasma levels before hemodialysis and 14–20 h after hemodialysis were correlated with the corresponding mean arterial pressure.

Key Words

Adrenomedullin
End-stage renal disease
Hemodialysis

Introduction

Human adrenomedullin (AM) is a 52-amino-acid peptide which was first isolated from pheochromocytoma tissue, but AM has also been found in other organs, as well as in plasma [1–3]. These notions suggest that AM may not only be a paracrine or autocrine peptide but also a circulating hormone. The physiological effects and pathobiological mechanisms of AM in man are not yet known. However, among the multiple pharmacological effects of AM observed in experimental animals, a major hypothesis has emerged that of a common effector of the coordinated systems by which fluid and electrolyte homeostasis and vascular tone are maintained [4–7]. Plasma levels of AM are increased in renal failure, and circulating AM correlates with plasma creatinine [8, 9]. The origins of the markedly

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elevated AM levels in end-stage renal disease, which are 2- to 5-fold higher than the levels in healthy subjects are not fully known. There is no doubt that decreased renal clearance of AM may contribute to the raised AM concentrations. However, circulating AM levels may differ substantially among patients with end-stage renal disease. Of note are the correlations between plasma AM levels and atrial natriuretic factor, both in congestive heart failure and chronic failure [10, 11], suggesting that volume expansion may be a stimulus for enhanced synthesis or secretion of AM in patients with end-stage renal disease. However, dialysis-induced contraction of extracellular fluid volume failed to alter AM levels in patients receiving hemodialysis [9].

The objectives of the present study were (a) to measure the predialysis AM levels in relation to coexistent diseases, (b) to establish correlations between plasma AM levels and corresponding predialysis volume-dependent blood pressure levels, and (c) to re-evaluate the effects of dialysis-induced volume contraction of postdialysis plasma AM concentrations.

Materials and Methods

Study Population

Fifty-eight patients with end-stage renal disease receiving regular hemodialysis were enrolled in the study (36 men, 22 women; mean age 61 ± 15 years, range 20–84 years). They all gave written consent to participate in these investigations, which were approved by the local ethical committee. The cause of chronic renal failure was glomerulonephritis in 16 patients, diabetic nephropathy in 14, nephrosclerosis in 5, tubulointerstitial nephritis in 5, polycystic disease in 4, systemic vasculitis in 2 and renal disease of unknown origin in 12. Twenty-four healthy subjects (14 men, 10 women; mean age 56 ± 16 years, age range from 22 to 68 years) served as controls. Twenty-one patients with end-stage renal disease had chronic arterial hypertension (blood pressure reading was consistently over 140/90 mm Hg), 20 patients suffered from coexistent heart failure (New York Heart Association II–IV). None of the patients had liver cirrhosis or chronic obstructive pulmonary disease.

Study Design

Predialysis Plasma AM in Patients with End-Stage Renal Disease. Prior to a regularly scheduled hemodialysis session, patients remained supine for 15 min before blood samples were taken from the arteriovenous fistula. Blood pressure and pulse rate were measured before blood samples were taken.

Effect of Hemodialysis on Plasma AM Levels. Hemodialysis was performed three times per week for 4–5 h with volumetrically controlled ultrafiltration devices (Fresenius MTS 2008 or 4008, dialysate flow 500 ml/min, blood flow rates of 200–250 ml/min). Fluid removal rates were adjusted to clinical needs. All patients underwent bicarbonate dialysis utilizing low-flux hemodialyzers (F6 low-flux polysulfone Fresenius, Bad Homburg, Germany, surface 1.25 m^2, UF coeff. 5.5 ml/h × mm Hg, urea clearance 183 ml/min, B_{12} clearance 56 ml/min). None of the patients received intradialytic fluid replacement or antihypertensive drugs. At the end of the dialysis session (immediately prior the backwash fluid substitution, postdialysis blood samples were taken in all 58 patients. In 16 of the study patients further blood samples were taken the morning after the hemodialysis session (14–20 h after hemodialysis).

Laboratory Methods. Blood was collected in tubes containing 1 mg/ml disodium EDTA and 500 U/ml aprotinin. Samples were centrifuged immediately at 4°C and the plasma was frozen and stored at –80°C pending the assay. Plasma levels of AM were measured by specific radioimmunoassay after extraction using C-18 Sep-Pak cartridges (AM-RIA Kit and cartridges from Phoenix Pharmaceuticals Inc., Mountain View, Calif., USA). The intra-assay and interassay coefficients of variation were <5 and 8%, respectively. The detection limit for the assay was 3.01 pg/ml, the half-maximal inhibition dose of radioiodinated binding by AM was 46 pg/tube. The maximal and minimal concentrations detected by the assay were 240 and 3.01 pg/ml, respectively. The radioimmunoassay had 100% cross-reactivity with human AM 1–52 and no cross-reactivity with AM 13–52 calcitonin gene-related peptide and amylin.

Statistical Analysis

Mean arterial pressure was calculated by the standard formula. Data are given as mean ± standard deviation (SD). Postdialysis AM concentrations were corrected for ultrafiltration. The results of the groups

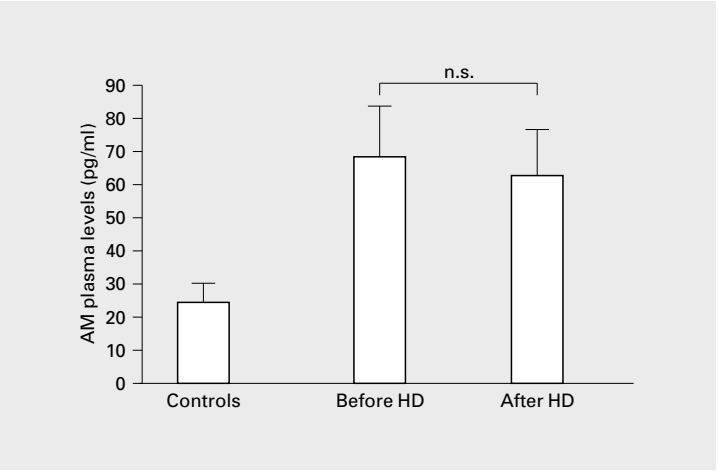

Fig. 1. Plasma concentrations of AM in controls and in patients with end-stage renal disease before and after hemodialysis (HD) (uncorrected for ultrafiltration) (mean ± SD); n.s. = not significant.

or subgroups were analyzed and compared by the Mann-Whitney U test and correlation tests. p values <0.05 were considered significant.

Results

The demographic characteristics of the patients with end-stage renal disease and healthy controls are given in table 1. There were no statistically significant differences between patients and controls with respect to age and body weight. Compared to age- and body-weight-matched healthy subjects, hemodialysis patients had significantly higher mean systolic and diastolic blood pressure values. Hemodialysis patients had significantly increased plasma AM levels (67.9 ± 15.4 vs. 27.1 ± 4.6 pg/ml; $p < 0.001$; fig. 1). There were significant differences in circulating AM between subgroups of hemodialysis patients. Plasma AM levels were 58.0 ± 13.2 pg/ml in normotensive patients on hemodialysis, 71.4 ± 15.4 pg/ml in hypertensive patients on hemodialysis, 74.3 ± 14.9 pg/ml in normotensive hemodialysis patients with heart failure and 76.8 ± 9.6 pg/ml in hypertensive hemodialysis patients with heart failure. There were significant correlations between systolic and mean arterial pressure and predialysis plasma AM concentrations in all patients studied (fig. 2).

Table 1. Basal clinical and laboratory data of HD-patients and controls

	Healthy subjects (n = 24)	Patients on HD (n = 58)
Age, years	56.2 ± 14.5	61.4 ± 15.8
Body weight, kg	68.8 ± 10.4	63.1 ± 12.5[a]
SAP, mm Hg	122 ± 18	147 ± 28[a]
DAP, mm Hg	70 ± 8	78 ± 14[a]
MAP, mm Hg	88 ± 11	101 ± 17[a]
Pulse rate min	70 ± 3	77 ± 6[a]
Serum creatinine, mg/dl	0.7 ± 0.2	7.5 ± 2.3[a,b]

Values are expressed as mean ± SD. HD = Hemodialysis; SAP = systolic arterial pressure; DAP = diastolic arterial pressure; MAP = mean arterial pressure. [a] $p < 0.05$ vs. healthy subjects; [b] $p < 0.05$ vs. prehemodialysis values.

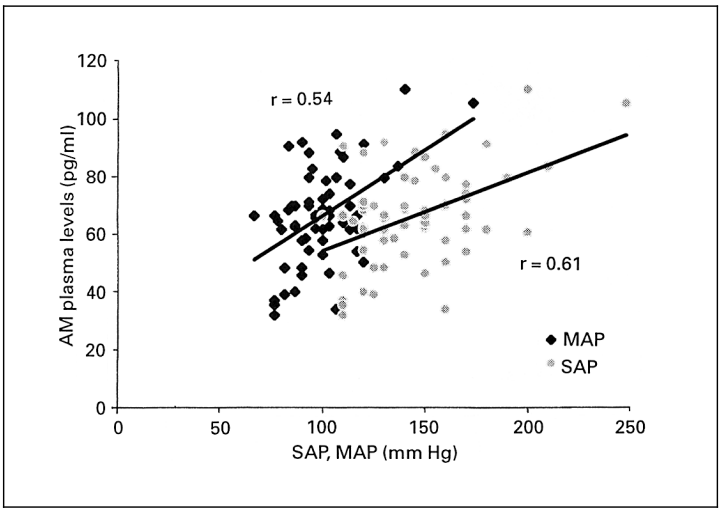

Fig. 2. AM plasma levels in patients with end-stage renal disease before hemodialysis (HD) in correlation with systolic arterial pressure (SAP) and mean arterial pressure (MAP).

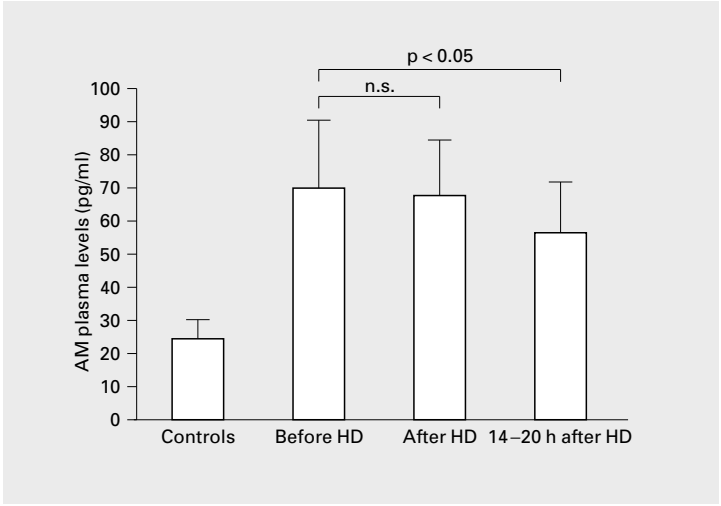

Fig. 3. Plasma levels of AM in a subgroup of 16 patients with end-stage renal disease before hemodialysis, after hemodialysis (uncorrected for ultrafiltration) and the next morning (14–20 h after hemodialysis); n.s. = not significant.

Hemodialysis-induced ultrafiltration resulted in a significant reduction of body weight (2.4 kg), a significant fall in blood pressure values and a significant rise in pulse rate. However, hemodialysis did not acutely affect plasma AM levels in our population of 58 patients receiving hemodialysis. Correction of postdialysis plasma AM for ultrafiltration-induced contraction of intravascular volume revealed a slight but not statistically significant rise in plasma AM as compared to predialysis patients. Despite a slight gain in body weight (0.9 kg) measurement of plasma AM 14–20 h after the hemodialysis session showed a significant reduction in plasma AM concentration as compared to predialysis or the first postdialysis plasma AM concentration even for corrected plasma AM concentration (table 2, fig. 3).

Table 2. Basal clinical and laboratory data of 16 patients investigated 14–20 h after HD

	Pre-HD	Immediately post-HD	14–20 h post-HD
Age, years	55.9 ± 19.2		
Body weight, kg	61.3 ± 11.4	58.9 ± 10.3[a]	59.8 ± 9.2
SAP, mm Hg	142.3 ± 22.2	134.6 ± 14.8[a]	137.8 ± 14.9[a]
DAP, mm Hg	86.2 ± 10.1	80.8 ± 9.0	84.4 ± 8.3
MAP, mm Hg	101.1 ± 17.7	94.5 ± 15.4[a]	97.9 ± 12.9
Pulse rate/min	78.9 ± 6.6	88.3 ± 9.1[a]	80.0 ± 7.7[b]
Serum creatinine, mg/dl	8.4 ± 3.2	4.1 ± 2.8[a]	5.6 ± 3.1[a,b]

Values are expressed as mean ± SD. [a] $p < 0.05$ vs. prehemodialysis values; [b] $p < 0.05$ vs. posthemodialysis values.

Discussion

The major result of our investigation demonstrates that the circulating AM concentration in patients with end-stage renal disease receiving regular hemodialysis may not be determined by a single factor, but may be the result of different interacting mechanisms. Confirming previous reports on plasma AM in end-stage renal patients, predialysis levels were significantly increased [8, 9]. To date, the mechanisms of circulating AM elevation in human chronic renal failure remain undefined. Synthesis, secretion, metabolism and clearance of AM have not been elucidated. However, AM may play a role in regulating water and electrolyte excretion by the kidney through release of endogenous NO [5]. Since other peptide hormones or small fragments of peptides are known to be metabolized in the kidneys or excreted by the kidneys into the urine, the possibility of a decreased clearance of AM in renal failure patients cannot be excluded as an explanation for the elevated AM observed in end-stage renal disease patients. In fact, other investigators reported that plasma AM concentrations are positively correlated with serum creatinine and inversely correlated with the glomerular filtration rate [9]. Sato et al. [12] characterized immunoreactive AM in human plasma and urine. In 5 normal subjects, immunoreactive urinary AM was found at 5–6 times the concentration present in plasma. Thus, AM found in the urine, may come from the plasma or be produced within the kidneys. However, circulating AM levels in our patients differed significantly between patients with uncomplicated end-stage renal disease and patients with coexistent cardiovascular disorders. The higher AM levels found in the latter subgroup of patients confirm previous reports of increased plasma AM levels in patients with congestive heart failure or arterial hypertension but normal renal function [9, 11, 13]. There is growing evidence that cardiac secretion of AM occurs in human heart failure. Of note is the observation that AM plasma levels correlate with parameters of the sympathetic nervous system [14, 15] as well as with markers of volume status [11].

The current study confirms that hemodialysis with or without ultrafiltration does not alter circulating AM levels, whether or not

plasma AM levels taken at the end of the dialysis session are corrected for volume concentration. There is no doubt that the peptide AM (molecular weight 6.028) poorly passes low-flux hemodialyzer membranes. However, Mallamaci et al. [16] provided preliminary evidence that isolated ultrafiltration may reduce plasma AM levels, and in our study late measurements of plasma AM revealed significantly lower AM levels in a subgroup of patients. Therefore secretion of AM seems to be increased in response to expansion of extracellular fluid volume overload in end-stage renal disease and may contribute to the high plasma AM levels. Dialysis-induced ultrafiltration may induce a counteracting stimulation of the sympathetic nervous system activity which may mask the effect of ultrafiltration on plasma AM levels.

Although AM levels are elevated in human diseases such as end-stage renal disease, direct cause and effect relationships have not been established. Moreover, clear definitions of the roles AM in health or kidney diseases are needed.

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