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Circulating adrenomedullin in cirrhosis: relationship to hyperdynamic circulation

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Background/Aims: **Peripheral arterial vasodilation may be the key factor in the sodium and water retention of cirrhosis. The mechanism responsible for this vasodilation remains to be fully elucidated. Adrenomedullin is a novel peptide, highly expressed in cardiovascular tissues, with potent and long-lasting vasodilating activity.**

Methods: **The possible implication of adrenomedullin in the hemodynamic changes of cirrhosis has been investigated. We measured the plasma concentration of adrenomedullin in 20 cirrhotic patients and 11 healthy subjects. In addition, systemic, portal and renal hemodynamics, hormonal factors and renal function parameters were evaluated in the same patients.**

Results: **Circulating adrenomedullin was significantly higher in the group of patients with cirrhosis (72.1; 46–100 vs 21.6; 11–34 fmol/dl, respectively; $p<0.02$) and was directly correlated with the Pugh score (r: 0.6; p: 0.01), inversely correlated with the creatinine clearance (r: −0.6; $p<0.01$) and tended to inversely correlate with systemic vascular resistance index (r: −0.46; p: 0.07). There were no portal-peripheral differences in adrenomedullin levels. Transjugular intrahepatic portosystemic shunt insertion did not induce changes in the peripheral concentration of adrenomedullin, but baseline values of this hormone predicted the degree of hyperdynamic circulation after TIPS.**

Conclusions: **Circulating adrenomedullin is increased in cirrhosis. These levels increase with the severity of the disease, especially in patients with hepatorenal syndrome. This peptide may contribute to vasodilation of cirrhosis.**

Key words: **Adrenomedullin; Cirrhosis; Peripheral vasodilation.**

Cirrhosis and portal hypertension are associated with high cardiac output and low systemic vascular resistance. These changes are more pronounced as liver disease progresses and may be secondary to peripheral vasodilatation, which in turn has been proposed as a key factor in initiating the sodium and water retention of cirrhosis (1).

The pathogenesis of the circulatory changes observed in cirrhotic patients has not been fully clarified, and while overproduction of nitric oxide (NO) may play a role (2–6), the predominant isoform of NO-synthase (constitutive or inducible) remains unsettled. Recently, a major role of the inducible calcium and calmodulin-independent isoform has been disputed (7–15). Conversely, the increased NO-synthase activity in cirrhotic rats seems to depend on the constitutive isoform (15). It has also been suggested that endothelial constitutive NO-synthase (e-NOS) up-regulation may be a consequence of pulsatile blood flow and shear stress on wall vessels (16). However, long-term inhibition of NO synthesis reversed the hyperdynamic circulation of cirrhotic rats (14). Circulating levels of potent vasodilating peptides that could contribute to the up-regulation of e-NOS, such as substance-P and CGRP are found to be elevated in cirrhotic patients (17,18). Hence, the study of new endothelium-depend-

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ent vasodilating peptides and their relationship to the extent of the hemodynamic disturbance of cirrhosis is warranted.

Adrenomedullin is a novel 52 amino acid peptide which shares slight homology with CGRP (19), originally isolated from human pheochromocytomas (20), and may be involved in the regulation of blood pressure. Endothelial cells are thought to be an important source of adrenomedullin (21). Most immunoreactive adrenomedullin is also expressed in adrenal gland, lung, cardiac atria and splanchnic organs (22). Adrenomedullin is capable of eliciting potent and long-lasting forearm vasodilation in human volunteers at relatively low doses (23) and shows functional antagonism to endothelin-I as a part of a complex regulatory system of the vascular tone (24). It has been shown that vasodilation caused by adrenomedullin but not by CGRP depends upon NO endothelial release (25). In addition, it is believed that adrenomedullin causes vasodilation by increasing the intracellular cyclic-AMP (19). Although increased plasma levels of adrenomedullin in cirrhosis have recently been reported (26–28), little is known about the relationship between plasma adrenomedullin values and the extent of the circulatory disturbance in this condition. This work was undertaken to determine plasma adrenomedullin levels in relation to the degree of hemodynamic disturbance and renal and hepatic function changes occurring in patients with cirrhosis.

Patients and Methods

Our study included 11 healthy subjects (control group) and 20 patients with histologically proven liver cirrhosis. Adrenomedullin was also determined in ten patients with chronic renal failure before and immediately after undergoing hemodialysis. Five of these patients had high blood pressure.

Patients with cirrhosis with at least one of the following criteria were excluded from the present study: active gastrointestinal bleeding or recent bleeding and unstable condition, severe hepatic encephalopathy that precluded cooperation, cardiopulmonary diseases, diabetes mellitus requiring insulin, arterial hypertension, bacterial infection, portal vein thrombosis and intake of propranolol or any other cardioactive drug, as well as diuretics or non-steroidal anti-inflammatory drugs (NSAIDs) at least 10 days prior to admission. Cirrhosis was of alcoholic origin in all cases, except in three patients with chronic infection due to hepatitis C virus.

Our series of patients included four patients of Child class A, four of Child B and 12 of Child C (29). Thirteen of the patients were males and seven females, and age was 55±8 years. Two of the patients manifested hepatorenal syndrome as defined by the diagnostic criteria recently set by the International Ascites Club (30). Six patients required transjugular intrahepatic portosystemic shunt (TIPS) insertion because of gastroesophageal variceal bleeding that recurred despite standard pharmacological and endoscopic treatment (three patients) and refractory ascites (three patients). The second hemodynamic study was performed 1 month after TIPS insertion in five patients and 6 months after TIPS in one. The second sampling for adrenomedullin determination was done at the same time as the second hemodynamic study.

The control group consisted of seven males and three females (age 49±4 years). The study also included ten sex- and age-matched patients with chronic renal failure. The protocol was approved by the Ethics Committee at the Hospital Xeral de Vigo and the Office of Human Subjects Research at the National Health Institutes in Bethesda, MD, USA. Written information was given to all patients and written informed consent was obtained from each participating patient.

Both controls and patients were placed on rest and on a 40–60 mmol/day sodium diet for 5 days. Diuretics, beta-blockers and other cardioactive drugs were withheld during this period of the study.

Protocol

The day after admission, samples of urine and venous blood were obtained for routine analytical studies. An electrocardiogram, chest film and abdominal ultrasonography were also performed. On the fourth day a blood sample was drawn and a 24-h urine sample was collected for renal function studies. On the fifth day the central venous pressure was recorded, as well as the heart rate and blood pressure. At 9.00 a.m. blood samples were drawn for plasma renin activity (PRA) and plasma aldosterone concentration (PAC) determination. Plasma samples for adrenomedullin determination were immediately centrifuged at 4°C and the supernatant was stored at −70°C until determination. PRA was determined by radioimmunoassay for Angiotensin I (Angiotensin radioimmunoassay test, Sorin Biomedica, 13040 Vercelli, Italy) and PAC by direct radioimmunoassay (Aldosterone II RIA diagnostic kit, Abbott Laboratories, Germany). Plasma adrenomedullin was determined in both peripheral venous blood and portal vein blood samples in five patients at the time of TIPS placement.

Radioimmunoassay (RIA) of adrenomedullin

Serum samples (1.0 ml) were mixed with an equal volume of 0.1% alkaline-hydrolysed-treated casein (31) in

phosphate-buffered saline (PBS), and extracted through C-18 Sep-Pak 400 mg cartridges (Waters Corp, Mildford, MA, USA). The proteins were eluted with 80% isopropanol and the recovered volume was freeze dried to eliminate the organic solvent. Extracts were reconstituted in 400 μl RIA assay buffer (10 M phosphate, 50 mM ethylenediamine tetraacetate, 135 mM NaCl, 0.05% Triton X-100, 0.1% Tween 20, 1% bovine serum albumin (BSA), 0.1% alkaline-hydrolysed-treated casein, 20 mg/l phenol red, pH 7.5), spun at 14 000 rpm for 10 min at 4°C to remove any solid matter, and three 100-μl aliquots from each sample were separated for analysis. The RIA was performed using the Phoenix human adrenomedullin RIA kit and following the manufacturer's instructions. Briefly, 100 μl of anti-adrenomedullin antibody and ^{125}I-adrenomedullin (15 000 cpm) were added to each sample and the mixture was incubated for 16 h at 4°C. After centrifugation at 3750 rpm for 30 min at 4°C, the supernatant was discarded, separation of bound and free tracer was accomplished using a 1:200 dilution of goat anti-rabbit IgG in a 6% PEG 8000 matrix, and the radioactivity in the pellets was measured in a 1277 Gammamaster instrument (Wallac, Gaithersburg, MD, USA). The radioactive counts were compared to a standard curve, and the concentration of adrenomedullin was calculated by linear regression. Recovery in the assay averaged 66% and the variation between assays was less than 10%. The displacement of tracer obtained by increasing volumes of serum extracts was parallel with that observed with the standard curve. The coefficient of variation intra-assay was 6% and the detection limit was 1.6 fmol/dl. The r-square for linearity was 0.990, the slope −2.6 and the B50 11.8 fmol/dl.

Hemodynamic calculations

In the fasting patients, on the fifth day, at 9.00 a.m. a pulsed Doppler echocardiographer was used (Vingmed 700 CMF, Horten, Norway, with a 3.5 MHz transducer) to determine the cardiac output from a Fourier-transformed left ventricle outflow curve, through an apical window, after bidimensional images were obtained. We found a fair correlation between the values of stroke volume and cardiac output as estimated by radionuclide ventriculography and the echo-Doppler method (n: 16: r: 0.8: p: 0.02. Fernández-Rodriguez CM, Penas J, Guitián R. Unpublished observation). To avoid interobserver variation, this determination was performed by the same observer throughout the study (J.P.). The coefficient of intra-observer variation was less than 5%. In addition, the results of this non-invasive technique have been shown to correlate with those obtained by the thermodilution method (32–34). A complete systemic hemodynamic study was performed on five healthy subjects. Since the apical window was considered inappropriate in three patients, complete systemic hemodynamic data were available from 17 patients. In six patients the hemodynamic study and hormonal determinations were repeated after TIPS insertion. The patency of TIPS was verified by Doppler sonography. The cardiac and systemic vascular resistance indexes were corrected for the body surface according to the ideal body area in patients with ascites and/or edema (35):

$$IBW=(H-100) - (H-150)/4$$

where H stands for height.

Statistical analysis

Results are presented as means and standard deviation, medians and ranges. The unpaired two-tailed t-test was used for comparison between means of normally distributed data. The Wilcoxon test and the Mann-Whitney U test were performed for nonparametric paired and unpaired comparison between two groups, respectively. Linear coefficients of correlation were also used in the analysis of the results. P-values less than 0.05 were considered significant. Since the distribution of the values of plasma adrenomedullin is not normal and the variances of data are not homogeneous (homoced-

TABLE 1

Hemodynamic, hormonal and renal function parameters in healthy subjects and cirrhotic patients

	Control	Cirrhotic	p
Nap (mEq/l)	140±1.4	136±1.5	N.S.
Ccr (ml/min)	128±17	71±8	<0.01
UNaV (mEq/24 h)	127±23.6	36±7.7	<0.01
MAP (mmHg)	80±4.1	82.7±9.1	N.S.
CVP (mmHg)	3±0.4	3.6±0.3	N.S.
SVRI ($dyn \cdot s^{-1} \cdot cm^{-5} \cdot m^{-2}$)	2105±170	1815±100	<0.05
CI ($l \cdot min^{-1} \cdot m^{-2}$)	2.7±0.19	3.8±0.2	<0.05
HVPG (mmHg)		18.3±4.9	
PRA ($ng \cdot ml^{-1} \cdot h^{-1}$)	0.6±0.26	9.3±3	<0.05
PAC (ng/ml)	126±36	1398±739	<0.05
Albumin (g/dl)	3.9±0.1	2.9±0.23	<0.01
Bilirubin (mg/dl)	0.925±0.25	3.5±0.1	<0.05
Prothombin index (%)	95±3.3	59±3.7	<0.01

Data as mean±SEM.

Nap: Plasma sodium; Ccr: Creatinine clearance (corrected for body surface area and expressed per 1.73 m^2); UNaV: Urinary excretion of sodium; MAP: Mean arterial pressure; CVP: Central venous pressure; CI: Cardiac index; SVRI: Systemic vascular resistance index; HVPG: Hepatic venous pressure gradient; PRA: Plasma renin activity; PAC: Plasma aldosterone concentration. N.S.: Non-significant differences.

astics), the Spearman rank correlation was used for the regression analysis.

Results

Values of hepatic, renal function, hormonal and hemodynamic parameters are summarized in Table 1. The plasma values of adrenomedullin in the group of patients were significantly higher than in the control group (median: 58.5 fmol/ml; range 23 to 205 fmol/ml vs 21.6 fmol/ml; range 11 to 34 fmol/ml, Fig. 1).

Patients with chronic renal failure showed higher adrenomedullin levels than subjects in the control group (median: 72.1; range 46.8–100 vs 21.6 range 11–34 fmol/ml respectively; $p<0.02$). In addition, adrenomedullin concentrations dropped in these patients after undergoing hemodialysis (median 72.1; 46.8–100.8 vs 52; 34.4–96.6, fmol/ml, respectively; Fig. 2). Consistent with these findings, the highest values were found in patients with hepatorenal syndrome (closed circles, Fig. 1). There were no differences in plasma adrenomedullin between renal patients with or without arterial hypertension.

In the cirrhotic patients, no correlation was found between adrenomedullin levels and the mean arterial pressure nor between adrenomedullin values and the hepatic venous gradient pressure. There was no correlation between adrenomedullin levels and plasma renin activity nor with plasma aldosterone concentration. Plasma levels of this peptide correlated inversely with creatinine clearance (Fig. 3) and correlated directly with the Pugh score (r: 0.6; p: 0.01). Adrenomedullin values tended to inversely correlate with systemic vascular resistance index, although without reaching statistical significance (r: −0.46; p:0.07).

Six patients were treated with TIPS. In these patients, the cardiac index increased after the procedure (3.75; 2.3–4.2 vs 4.2; 2.2–6.7 $l \cdot min^{-1} \cdot m^{-2}$, respectively; $p<0.05$). However, plasma adrenomedullin concentration was similar before and after TIPS (median: 38.8; range 10.1–205.2 vs 38, range 35.4–49.8 fmol/ml, respectively; p: 0.27). We also found that the concentration of adrenomedullin in the portal vein was similar to that found in the peripheral circulation (54.1±19 vs. 53.41±46; n: 5; N.S.). Interestingly, the baseline adrenomedullin levels predicted the intensity of hyperdynamic circulation which developed in the cirrhotic patients after TIPS insertion, since the baseline plasma

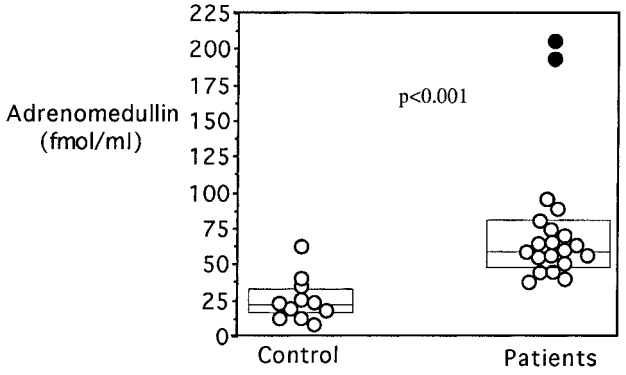

Fig. 1. Individual values of plasma adrenomedullin in the group of patients with cirrhosis and the control (median: 58.48; range 23.2 to 205.2 fmol/ml vs 24.1; range 11 to 34 fmol/ml, respectively). Boxes represent median values and the interquartile ranges and the closed circles those patients with hepatorenal syndrome. (Non-parametric Mann-Whitney U test.)

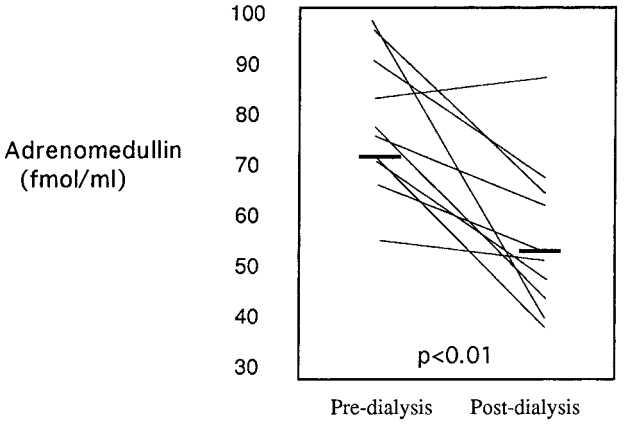

Fig. 2. Plasma levels of AM in patients with chronic renal failure prior to and after undergoing hemodialysis. Horizontal lines represent the median values (72.1 vs 52 fmol/ml, respectively). (Non-parametric Wilcoxon test.)

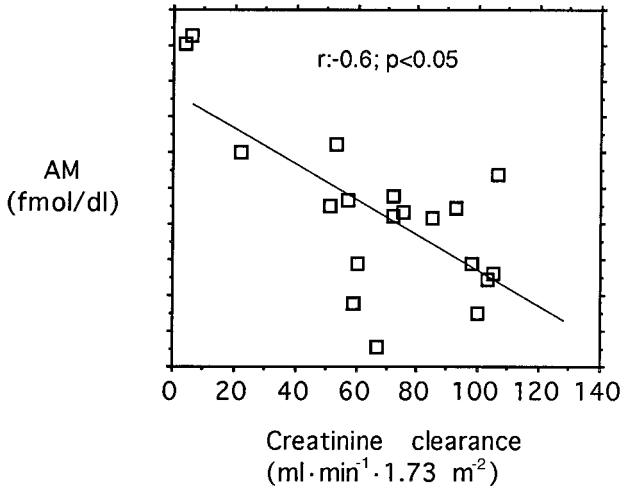

Fig. 3. Inverse correlation between the plasma AM concentration and the creatinine clearance in the group of patients. (Non-parametric Spearman rank correlation.)

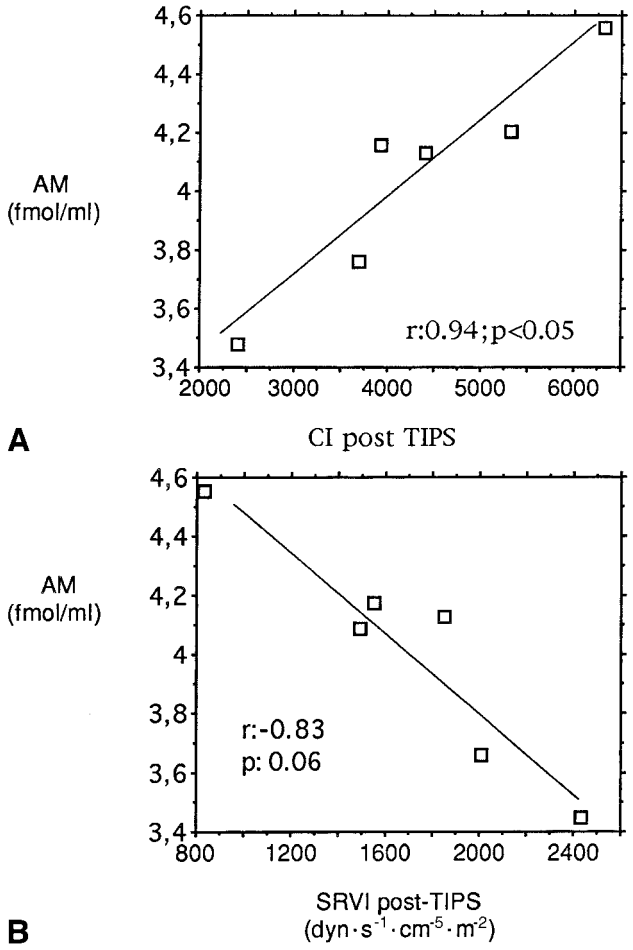

Fig. 4. A. Correlation between the plasma AM concentration and the cardiac index (CI) after transjugular intrahepatic portosystemic shunt (TIPS) insertion patients with cirrhosis. (Non-parametric Spearman rank correlation.). B. Inverse correlation between the plasma AM levels and the systemic vascular resistance index after TIPS. (Non-parametric Spearman rank correlation.)

adrenomedullin levels directly correlated with the cardiac index after TIPS (Fig. 4A), and there was a nearly significant inverse correlation with the systemic vascular resistance index (SVRI) after TIPS placement (Fig. 4B).

Discussion

Our findings confirm previous observations on the presence of hyperdynamic circulation in liver cirrhosis (36–38), and show increased plasma levels of adrenomedullin-like immunoreactivity in patients with cirrhosis as compared with healthy controls. Cheung & Leung reported increased adrenomedullin levels in different conditions including liver cirrhosis (27). The absolute values of plasma adrenomedullin reported by these authors were different from the values obtained in the present study. These differences may be due to the different radioimmunoassay and different antibodies used. The relative changes in adrenomedullin, observed in patients with cirrhosis, are similar in both studies.

In our patients the plasma levels of this vasodilator correlated directly with the Pugh score and inversely with creatinine clearance. It can be reasoned that reduced hepatic or renal clearance, or both, could account for our results. Nevertheless, our results on the portal-systemic gradient indicate that there is no net hepatic clearance of adrenomedullin in patients with cirrhosis. We did not measure the renal arteriovenous concentration of adrenomedullin. However, the marked reduction in plasma adrenomedullin concentration after hemodialysis in patients with chronic renal failure suggests that a defective renal clearance might contribute to the increased levels observed in those patients with chronic renal insufficiency or with hepatorenal syndrome. An increased adrenomedullin production to counterbalance plasma volume overload might be an additional mechanism accounting for increased adrenomedullin plasma concentration in renal patients (39).

An increased adrenomedullin production in cirrhosis may account for the elevation of plasma adrenomedullin levels in our patients. Substances such as tumor necrosis factor-α (TNF-α) or endotoxin are reported to be increased in cirrhosis (40–43) and it has been shown that TNF-α markedly stimulates the production and secretion of adrenomedullin by vascular smooth muscle cells (VSMCs) (44). Therefore, increased adrenomedullin activating substances such as mild endotoxemia and/or increased circulating levels of TNF-α, as observed in advanced liver diseases (40–43), could enhance adrenomedullin synthesis, thus increasing adrenomedullin-mediated NO production. In addition to these compounds, several vasoactive compounds such as substance-P, endothelin-I and adrenalin, all of which have been found to be increased in the peripheral circulation of cirrhotic patients (17,45,46) are capable of stimulating adrenomedullin production by VSMCs (47).

Although the correlation did not reach conventional statistical significance, adrenomedullin levels tended to correlate inversely with the systemic vascular resistance index, which suggests that this peptide might participate in the hemodynamic disturbance of cirrhosis. In our study, the SVRI of those patients with advanced disease may have been overestimated, as it was calculated from body surface area according to the Lorentz formula. It has been suggested that this formula may

overestimate weight in wasted patients with ascites and edema (35).

It is interesting that plasma values of adrenomedullin predicted the intensity of further vasodilatation in patients after TIPS placement. It has been suggested that activation of endogenous vasoconstrictor forces may offset or attenuate the effect of vasodilating substances (17). After TIPS insertion, a partial deactivation of the vasoconstrictor forces occurs (48), because of either increased replenishment of the central vascular compartment or alleviation of the intrasinusoidal pressure. Therefore, TIPS may render vasodilatory activity unopposed. Thus, the higher the plasma adrenomedullin levels pre-TIPS, the more intense post-TIPS vasodilation would be. On the other hand, the lack of correlation between the adrenomedullin concentration and the hepatic venous pressure gradient suggests that intrasinusoidal hypertension does not play a significant role in the elevation of plasma adrenomedullin. In fact, adrenomedullin levels did not change after reduction of sinusoidal hypertension. The lack of effect of TIPS on plasma levels of vasodilating peptides has also been shown in the case of substance-P (17).

In addition to adrenomedullin, other humoral factors causing endothelial-dependent vasorelaxation have been found to be increased in cirrhosis (17,18). Whether or not these compounds might have a synergistic action in the up-regulation of e-NOS, observed in cirrhotic rats (49) requires further research.

In summary, circulating adrenomedullin is increased in cirrhosis, especially in patients with poor hepatic and renal function and more severe peripheral arterial vasodilation. Plasma levels of adrenomedullin seem to accurately predict the development of hyperkinetic circulation after the placement of TIPS. It is concluded that this potent vasodilator may be involved in the peripheral vasodilation of cirrhosis.

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