Nephrol Dial Transplant (1989) 4: 222–227

Nephrology Dialysis Transplantation

Original Article

Comparative Effects of Haemodialysis and Haemofiltration on Plasma Atrial Natriuretic Peptide

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Abstract. The effects of 4 h haemodialysis (15 patients) or 4 h haemofiltration (five patients) on plasma concentrations of atrial natriuretic peptide (ANP) were compared by means of a sensitive radioreceptor binding assay, and related to accompanying changes in body weight, blood pressure and plasma renin activity. Before dialysis, plasma ANP concentrations were considerably elevated: haemodialysis group 10–484 pmol/l (mean 156 pmol/l); haemofiltration group 72–320 pmol/l (mean 170 pmol/l). Although plasma concentrations of ANP fell markedly with treatment in both groups: post-haemodialysis 2–187 pmol/l (mean 67 pmol/l); post-haemofiltration 47–135 pmol/l (mean 79 pmol/l), after treatment it remained above the normal range in 14 of the 20 patients. Pretreatment plasma ANP was related to systolic blood pressure ($r = 0.459$; $P < 0.05$) but bore no relationship to mean or diastolic blood pressure, or plasma renin activity. The fall in plasma ANP concentration during treatment correlated with the postural blood pressure drop after dialysis ($r = 0.505$; $P < 0.05$), but was unrelated to changes in weight or plasma renin activity with haemodialysis or haemofiltration. Plasma ANP concentrations rose rapidly again in the 60 min after dialysis treatment, without change in body weight.

These results show that high levels of biologically active ANP circulate in end-stage renal disease. The fact that these are not reduced to normal by haemodialysis or haemofiltration, despite restoration to normovolaemic or hypovolaemic state, suggests that the increased levels of ANP in end-stage renal failure are due to both hypervolaemia and other factors, which may include occult cardiac dysfunction and loss of renal clearance.

Key words: Atrial natriuretic peptide; Haemodialysis; Haemofiltration; Renal failure

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Introduction

The primary stimulus for the cardiac release of atrial natriuretic peptide (ANP) is increased stretch of atrial myocytes [1], which is usually a consequence of increased atrial pressure due to either increased circulating plasma volume [2,3] or to congestive heart failure [4]. Several studies employing radioimmunoassay have confirmed that plasma concentrations of immunoreactive ANP are considerably elevated in patients with end-stage renal disease [5–10] and may be reduced by haemodialysis [6,7] or ultrafiltration [7,8,10]. The conclusion has been drawn that raised concentrations of ANP in end-stage renal disease are entirely due to plasma volume overload [5,10], which may be corrected by dialysis or ultrafiltration. However, the kidney is an important site of ANP clearance [11], and ANP may be metabolised in the renal tubule [12], in common with other peptide hormones [13]. Furthermore, cardiac function is often impaired in patients with renal failure, even when overt clinical cardiac failure is not apparent [14].

It is possible, therefore, that loss of normal renal clearance and occult cardiac dysfunction might account in part for the elevated plasma concentrations of ANP in end-stage renal disease. It is also unclear whether the high

plasma concentrations of immunoreactive ANP reported in renal failure truly represent biologically active peptides, as apparent elevations of peptide hormones in end-stage renal disease may be due to immunoreactive circulating peptide fragments. We have therefore measured plasma concentrations of ANP in patients with end-stage renal disease, employing a sensitive and specific radioreceptor assay for ANP, which detects intact biologically active peptide [15]. The changes in plasma ANP produced by haemodialysis and haemofiltration have been compared, and related to degree of volume depletion after dialysis assessed by weight change, postural blood pressure fall, and change in plasma renin activity.

Materials and Methods

Patients and Protocol

Twenty patients were studied, 15 aged 41–68 years (mean 55 years) undergoing conventional haemodialysis and five patients aged 49–71 years (mean 55 years) treated by haemofiltration. A description of the patients is given in Table 1. There were no significant differences in predialysis weight, blood pressure (measured by mercury sphygmomanometer) or plasma renin activity. No patient had clinical evidence of cardiac failure. All were taking phosphate binders; for hypertension two haemodialysis and three haemofiltration patients were receiving beta-blockers, and three haemodialysis patients and one haemofiltration patient were receiving nifedipine.

Table 1. Patient data

	Haemodialysis	Haemofiltration
Patients (F:M)	15 (14:1)	5 (4:1)
Mean age (range) years	55 (41–68)	55 (49–71)
Aetiology		
glomerulonephritis	1	2
pyelonephritis	4	1
analgesic	5	2
polycystic	3	
hypertension	2	
Predialysis		
weight (kg)	70±5	65±7
mean BP (mmHg)	101±4	107±5
PRA (ng/ml per h)	7.3±2.3	12.5±6.8

All patients were treated for 4 h, at the same time of day. They remained supine and did not eat or drink during treatment. Haemodialysis was carried out (15 patients) using a Gambro AK-10 machine and Gambro GF-120 H capillary dialysers. Haemofiltration was performed using a Gambro haemofiltration module and Gambro F-88 haemofilter. The dialysate contained; acetate 35 mmol/l; Na^+ 135 mmol/l; K^+ 1–4 mmol/l; Ca^{2+} 1.625 mmol/l; Mg^{2+} 0.25 mmol/l. The haemofiltration substitution solution contained lactate 35 mmol/l; Na^+ 136 mmol/l; K^+ 0 or 2 mmol/l; Ca^{2+} 2 mmol/l and Mg^{2+} 1.0 mmol/l. Body weight and supine and standing blood pressure (diastolic phase 5) were measured immediately before and at the end of the treatment period. At the commencement and termination of dialysis treatment, 4–6 ml blood samples were drawn from the arterial dialysis line into chilled tubes containing EDTA with the addition of aprotinin (0.1 mg/ml) for measurement of ANP, and into chilled tubes containing EDTA for the measurement of plasma renin activity. Three haemodialysis patients were studied again on a second day, when blood for ANP measurement was taken before dialysis, after 30 min and after 180 min dialysis treatment, and again 60 min after treatment had ended.

After immediate centrifugation at 4°C, plasma was stored at −20°C until ANP measurement, which was carried out by a radioreceptor assay described in detail elsewhere [4,15], after extraction of ANP from plasma by means of Sep-pak C18 cartridges. In brief, the radioreceptor assay involves competition of endogenous ANP in plasma, or alpha-human ANP standard, with a synthetic 24-amino acid radioligand (^{125}I tyr$_0$atriopeptin II) for ANP receptors in bovine adrenal cortex membranes. Assay tubes contained 50 µl of plasma extract resuspended in assay buffer, in a total volume of 200 µl, and were incubated at 25°C for 60 min, after which bound and free radioligands were separated by rapid-filtration assay with use of Whatman GF/C glass-fibre filters, whose radioactivity was measured in a gamma counter. The detection limit of the assay was 0.6 fmol per tube, intra-assay CV was 8.2% and inter-assay CV was 10.1%. Plasma ANP concentrations were corrected for recovery during the extraction procedure. Plasma renin activity was measured by radioimmunoassay [16].

All patients gave informed consent to the study, which was approved by the Hospital Ethical Committee. Results are expressed as mean±SEM. Statistical comparisons were made using the Wilcoxon test for paired data, and linear regression was by the method of least squares.

Results

In comparison with plasma ANP concentrations measured by radioreceptor assay in healthy controls (17±SD 13 pmol/l; $n=60$) [4], plasma ANP concentrations before dialysis treatment were consistently elevated in both the haemodialysis patients (10–484 pmol/l) and haemofiltration patients (72–320 pmol/l). The plasma peptide concentration fell significantly during treatment to a similar extent in both groups (Fig. 1; haemodialysis from 156 to 67 pmol/l; $P<0.01$, haemofiltration from 170 to 79 pmol/l; $P<0.05$). Weight loss was 2.2±0.2 kg

during haemodialysis treatment and 1.8 ± 0.3 kg during haemofiltration. Plasma renin activity increased during treatment, from 7.3 ± 2.3 to 13.1 ± 4.7 ng/ml per h in haemodialysis patients, and 12.5 ± 6.8 to 18.0 ± 10.7 ng/ml per h in haemofiltration patients. Mean supine blood pressures did not change during treatment; haemodialysis from 101 ± 4 to 101 ± 5 mmHg, haemofiltration from 107 ± 5 to 103 ± 7 mmHg.

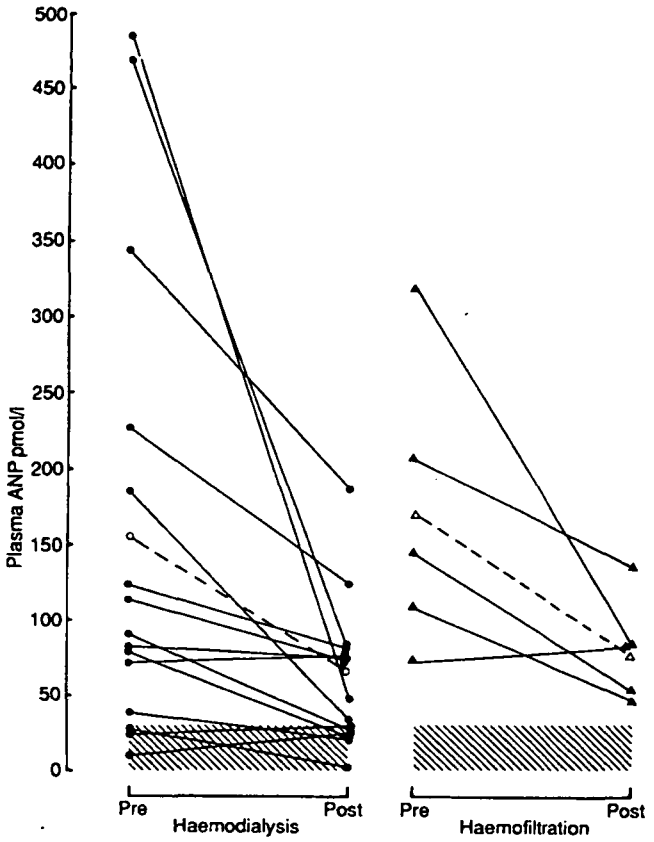

Fig. 1. Plasma ANP concentrations measured by radioreceptor assay before and after 4 h haemodialysis (●) or haemofiltration (▲) treatment. Significance of effect of treatment was $P<0.01$ (haemodialysis) and $P<0.05$ (haemofiltration) by Wilcoxon test. Open symbols show mean changes. Hatched area shows normal range for plasma ANP.

Predialysis plasma ANP concentration was related to predialysis systolic blood pressure ($r=0.459$; $P<0.05$), but not to mean blood pressure ($r=0.389$) or diastolic blood pressure ($r=0.279$), nor to plasma renin activity. No relationships were observed between change in plasma ANP concentration during dialysis treatment and fall in weight ($r=0.158$), change in plasma renin activity ($r=0.190$), or change in blood pressure ($r=0.219$) during the treatment period. However, a significant linear correlation was observed between change in ANP during dialysis treatment and degree of postural blood pressure decrease after dialysis (Fig. 2) such that those patients with the greatest reduction in ANP tended to have the greatest decrease in blood pressure after dialysis.

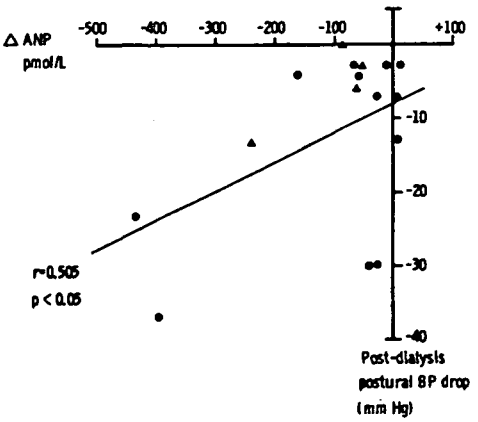

Fig. 2. Correlation between postdialysis postural blood pressure fall (erect mean BP - supine mean BP) and change in plasma ANP concentration during haemodialysis or haemofiltration treatment. Symbols as in Fig. 1.

Mean plasma ANP concentrations in the three patients studied sequentially during a 3 h haemodialysis treatment are illustrated in Fig. 3. ANP fell rapidly during the first 30 min of dialysis, and attained a level of 114 pmol/l after 3 h treatment. After haemodialysis was stopped, plasma ANP concentrations recovered rapidly to 83% of the predialysis value. Weight loss during treatment in the three patients was 2.2 ± 0.4 kg, with no significant change in the 60 min following dialysis.

Discussion

This study shows that plasma concentrations of biologically active ANP are greatly elevated in end-stage renal disease. These data confirm and extend previous reports employing radioimmunoassay [5–10] which demonstrated increased concentrations of immunoreactive peptide. Previous reports have also shown that there is a reduction in immunoreactive plasma ANP with haemodialysis [6,7] or ultrafiltration [7,8,10]. The present report demonstrates that biologically active ANP is reduced by haemofiltration, and that the reduction is comparable to that achieved by haemodialysis.

Although there is general agreement that plasma ANP concentrations are reduced during dialysis procedures, it remains uncertain whether these changes in plasma ANP are due to volume reduction, fall in atrial pressure, clearance of the peptide, or a combination of these three factors. The reduction of plasma ANP by dialysis has been taken by some to imply that ANP concentrations are raised in end-stage renal disease purely as a consequence of volume overload [5,10]. In support of this, there are some reports of reduction of ANP to the normal range by haemodialysis. However, these studies have employed direct radioimmunoassay without prior extraction of ANP from plasma [9,10], and it is established that direct

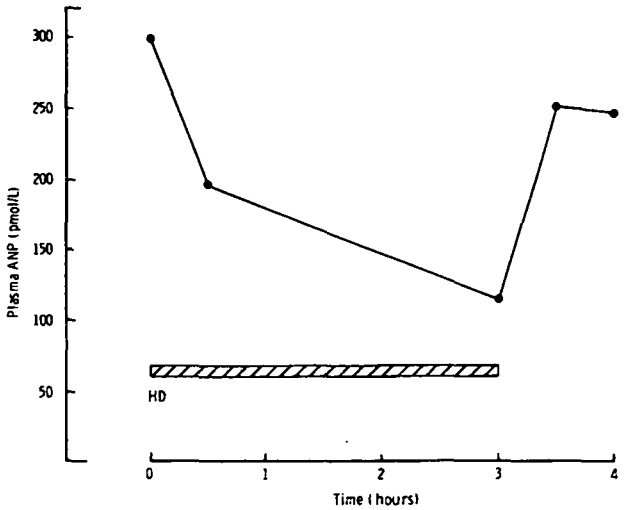

Fig. 3. Sequential plasma ANP concentrations in three patients before, during, and after 3 h haemodialysis treatment. Mean plasma ANP concentration is shown.

plasma radioimmunoassay of ANP is inaccurate at plasma ANP concentrations below 70 pmol/l [17]. In the present study, as in previous work employing extraction of ANP before assay (5–7) plasma levels were still well above the normal range after dialysis treatment, despite evidence of hypovolaemia, such as decrease in postural blood pressure. Similarly, in the study of Walker et al [18], plasma ANP levels remained in the range 20–120 pmol/l despite intensive ultrafiltration producing a 3 kg weight loss.

Blood pressure in patients with end-stage renal disease is usually volume-dependent, and in this respect a relationship was present between systolic blood pressure and plasma ANP concentration, as observed in previous studies [18]. The correlation was less marked in the present study than in previous reports; this may be due to the use of beta-blocker therapy in five of the 20 patients, since beta-blockade may result in a doubling of plasma ANP concentrations [19]. A correlation was also observed (Fig. 2) between reduction in ANP and decrease in postural blood pressure after dialysis, suggesting a degree of association between ANP reduction and volume depletion during dialysis. However, the correlation was not strong, and there was no relationship between reduced ANP concentration and reduction in weight or change in plasma renin activity, implying that other factors may also contribute to the fall in ANP during dialysis.

Several lines of evidence indicate that the kidney is an important site of ANP clearance. In man there is a large arteriovenous gradient for ANP across the kidney [11], and acute nephrectomy in rats results in a doubling of plasma ANP half-life [20]. There is evidence for metabolism of ANP in the renal tubule, and recent reports suggest that ANP is specifically degraded by kidney neutral endopeptidase [21]. In addition, Maack and colleagues have described the existence of biologically silent clearance receptors for ANP within the kidney, whose blockade results in increased plasma ANP levels [22]. Consistent with a role for the kidney in clearance of ANP from plasma, in patients with chronic uraemia but not on maintenance dialysis an inverse relationship has been described between plasma ANP and glomerular filtration rate, but not between ANP and blood volume [8], suggesting that impaired renal removal of ANP contributes to high plasma levels in uraemia, as is the case for other polypeptide hormones [13]. It is also conceivable that elevated ANP concentrations in chronic renal failure might in turn lead to down-regulation of ANP clearance-receptors outside the kidney, further increasing ANP plasma levels.

Since the kidney appears to be important in the metabolic clearance of ANP, it is possible that plasma clearance of ANP occurring during dialysis treatments might also contribute to the decrease in ANP concentration. Haemofiltration is more effective than conventional haemodialysis at clearing substances of high molecular weight, up to 10 000 daltons [23], and it would therefore be predicted that for ANP, with a molecular weight of 3082, haemofiltration would provide a greater clearance. However, this was not apparent from the present study, which showed that plasma ANP was reduced during both treatments to a similar extent. A recent study [24] has confirmed there is significant though not major clearance of ANP by both haemodialysis (25 ml/min) and haemofiltration (46 ml/min), compared with an overall metabolic clearance rate for ANP in end-stage renal failure patients of 1.04 l/min [25].

In addition to decline in clearance by the kidney, a further reason for the increase in plasma ANP concentrations seen in chronic renal failure may be impairment of cardiac function, independent of fluid volume status. Increase in atrial pressure, atrial distension, and consequent cardiac ANP release may occur as a consequence of acute volume expansion [26], with normal cardiac function, or when cardiac function is impaired, without significant volume overload [4]. Cardiac failure is common in end-stage renal disease, and recent studies have shown that right and left ventricular end-diastolic pressures at rest and after exercise are often elevated early in chronic renal failure, even when patients have no clinical evidence of congestive heart failure [14], as in the present study. Hence, occult cardiac dysfunction might contribute both to elevated plasma ANP concentrations in chronic renal failure, and to their decrease after dialysis, as haemodialysis may itself improve ventricular function [27].

The increase in ANP observed immediately after dialysis while weight remained unchanged in a subgroup of three patients also argues against simple volume depletion

being the sole factor causing ANP reduction during dialysis. Both this rapid increase in ANP and the early decrease at the commencement of treatment (Fig. 3), may be due in part to clearance of the peptide by the dialysis procedure, or to fluid redistribution between the extracellular and intravascular compartments, resulting in rapid changes in atrial pressure. Right atrial pressure may decrease dramatically during haemodialysis [24], but the equivalent changes in atrial pressure during the recovery phase after dialysis are not established.

The concentrations of biologically active ANP in end-stage renal failure observed in this study are well within the range required to cause significant arterial vasodilatation in man [28], and it is possible therefore that ANP may play an important and hitherto unsuspected role in determining vascular resistance in dialysis patients, and thus in their blood pressure regulation. Nevertheless, it is likely that vascular tone in these patients will ultimately depend on a complex interaction between ANP and other endogenous vasorelaxant mechanisms, and compensatory activation of the sympathetic nervous system and renin–angiotensin system, determined by plasma volume changes. In the present study, for example, plasma ANP concentrations decreased during dialysis, plasma renin activity increased, but no overall change was observed in blood pressure. Specific antagonists to ANP will be needed in order to determine the relative importance of its contribution to circulatory homeostasis, especially since reflex effects have been shown to override direct vasodilatory actions of ANP during infusion of the peptide [29].

In conclusion, the present study confirms that plasma concentrations of biologically active ANP may be considerably elevated in end-stage renal disease. They are reduced by haemodialysis or haemofiltration, but usually remain above the normal range despite fluid volume reduction to normal levels or below. Taken together with the lack of close relationship between decrease in ANP and indices of volume depletion (weight loss, fall in blood pressure), this implies that factors other than simple volume overload contribute to elevation of plasma ANP in chronic renal-failure patients, and its reduction with dialysis. These factors may include ANP clearance by the kidney or dialyser, clinically unsuspected cardiac dysfunction, or rapid fluid shifts between the intravascular and extravascular compartments.

Acknowledgements. AEGR was supported by a Medical Research Council Travelling Fellowship. The assistance of the Haemodialysis Unit staff of the Kantonsspital, Basel, in gathering data is gratefully acknowledged.

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Received for publication 18.4.88
Accepted in revised form 17.11.88