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Acute and long-term changes in plasma levels of atrial natriuretic factor in patients with renal replacement therapy

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Abstract. In 14 patients on hemodialysis who received kidney grafts from living related donors, plasma levels of immunoreactive atrial natriuretic factor (Ir-ANF) were determined in a sequence covering the last hemodialysis treatment, the day of transplantation, and a follow-up period of 6–12 months. The geometric mean value before dialysis was 196 pg/ml, the range 32–634. Weight loss during dialysis was 1.5 ± 1.1 kg (mean ± SD), but only a nonsignificant reduction in Ir-ANF levels occurred. On the day of transplantation, plasma Ir-ANF levels increased from 143 pg/ml before to 391 post-transplantation ($P = 0.02$, $n = 12$), probably in response to deliberate volume expansion. Post-transplant Ir-ANF levels correlated significantly to diuresis during the first 24 h, which ranged from 3.7 to 17.8 l (mean 6.6; $r = 0.65$, $P = 0.02$). On day 2, mean 24 h diuresis decreased to 3.3 ± 1.4 l. Most patients had reached their true dry weight by day 5, but Ir-ANF levels remained high, the geometric mean being 180 pg/ml. During further follow-up and preserved graft function (GFR range 34–88 ml/min per 1.73 m^2 body surface area), Ir-ANF levels declined to a geometric mean of 63 pg/ml by 2–6 months and to 36 at 12 months post-transplant. We conclude that plasma Ir-ANF levels are chronically elevated in patients with chronic renal failure but may be further stimulated by acute overhydration. Transplanted kidneys initially respond to the increased levels but adapt within a day. Even with good graft function, normalization of plasma Ir-ANF requires several weeks or months.

Key words: Atrial natriuretic factor, in renal transplantation – Kidney transplantation, atrial natriuretic factor

The atrial natriuretic factor (ANF) is released mainly from the right atrial myocytes in response to mechanical distention [3, 19]. This peptide has an integrative role in body fluid and electrolyte homeostasis by virtue of its diuretic/natriuretic functions [6, 11]. Several clinical studies have been carried out to clarify its role in patients with chronic renal failure, especially in relation to their fluid state [1, 4, 13–15, 18, 21–23]. Investigations have also been performed in renal transplant patients [4, 16, 20, 24] relating levels of endogenous or synthetic ANF to renal function. The present study was performed in patients on chronic hemodialysis undergoing transplantation with kidneys from living related donors. The objective was to monitor plasma immunoreactive ANF (Ir-ANF) levels over the last hemodialysis session, on the day of transplantation, and during further follow-up, and to relate these values to fluid balance and transplant function.

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Patients and methods

Fourteen hemodialysis patients undergoing renal transplantation with grafts from living related donors were investigated, 2 females and 12 males. The age range was 17–60 years. Seven had chronic glomerulonephritis, three polycystic kidney disease, two diabetic nephropathy, one systemic vasculitis, and one nephrosclerosis. The donors had undergone a series of investigations ascertaining normal renal function, including urinary concentration capacity and plasma clearance of ^{51}Cr EDTA after single bolus injection [5] as a measure of glomerular filtration rate (GFR). Data are shown in Table 1.

All transplantations were carried out in the morning, with the last planned hemodialysis treatment given on the previous day. One liter of saline was given intravenously from early morning until completion of the vascular anastomoses. As the circulation was reestablished, volume expansion was achieved by a bolus infusion of 200 ml mannitol and a second liter of saline for 15–30 min. In addition, dextran 40 (50 g in 500 ml of saline) was infused over a period of 6–8 h, starting perioperatively.

All grafts produced urine promptly following reestablishment of circulation. Diuresis was monitored and further intravenous infusion of saline and electrolytes given to compensate for losses. Furosemide was given to patients with slow diuresis. Dextran 40 was infused on days 2 and 3 as well. Weight shifts, diuresis, and serum creatinine values were monitored. Except in case 5, who died of pneumonitis, GFR was measured as in the donors at 6 months and/or 12 months post-transplant and was 51 (range 34–88) ml/min per 1.73 m^2 body surface area.

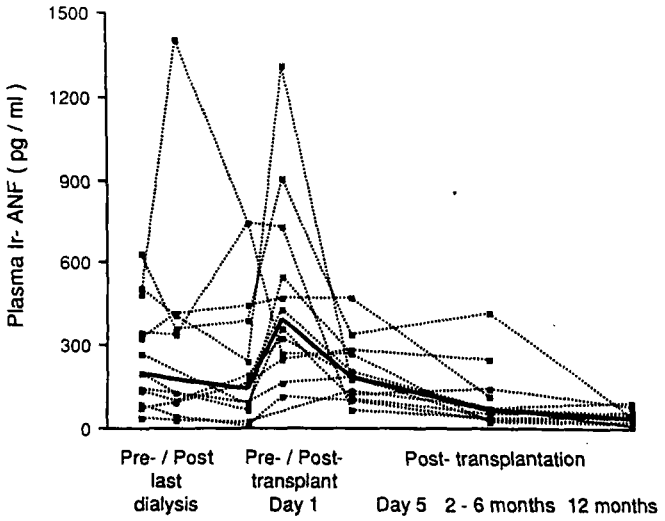

Fig. 1. Plasma levels of immunoreactive atrial natriuretic factor (Ir-ANF) in 14 subjects with end-stage renal failure who received renal transplants from living related donors. *Dotted lines* indicate changes over time in each individual. *Solid line* joins the geometric mean values

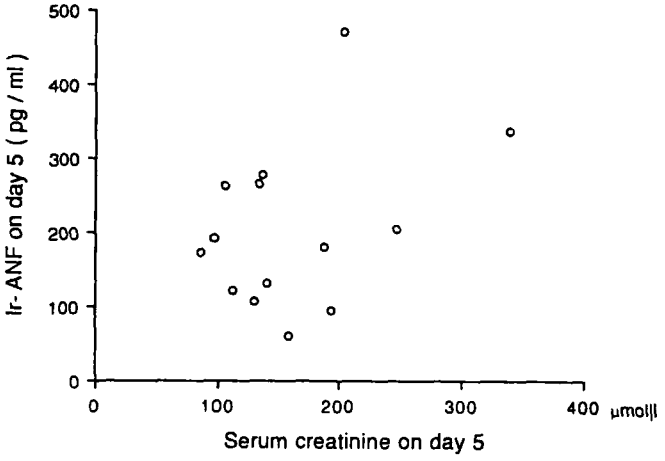

Fig. 2. Plasma levels of immunoreactive atrial natriuretic factor (Ir-ANF) on day 5 post-transplant in relation to concomitant serum creatinine values in 14 recipients of renal transplants from living related donors. $r = 0.37$ (NS)

Venous plasma for determination of Ir-ANF was sampled on five to seven occasions: immediately before and after the last hemodialysis session on the day before transplantation, in the morning before and in the afternoon following transplantation, on day 5, and 2–6 months and 1 year post-transplantation. The samples were collected in precooled EDTA-coated glass tubes, immediately cold centrifuged (+ 4°C), and then stored at − 70°C until analysis.

Human alpha ANF was extracted from EDTA plasma (2 ml) by passage under reduced pressure through a BondElut C 18 cartridge (Analytichem International) that had been pretreated by washing with 4 ml methanol and rinsed with distilled water. The plasma was acidified with 0.5 M HCl (5:1) and, after sample application, the cartridge was washed with 10 ml 0.1% trifluoroacetic acid (TFA) and 5 ml of a mixture containing 10% methanol and 0.1% TFA. The cartridges were then eluted with 2 ml of a mixture containing 75% acetonitrile and 25% TFA (0.1%), evaporated under a stream of nitrogen at 55°C, and dissolved in 0.5 ml phosphate triton buffer. The samples were then stored at − 25°C until radioimmunoassay.

A specific radioimmunoassay method was used for analysis of Ir-ANF. A rabbit antiatrial natriuretic peptide serum (RAS 8798, Peninsula Laboratories, Belmont, Calif) was used. Crossreactivity was 100% with rat ANF 1–28 and rat atriopeptin III, 90% with human ANF 8–23, 57% with human ANF 18–28, 50% with rat ANF 13–28, 27% with rat atriopeptin II, and 3% with rat atriopeptin I, but there was no reactivity with Arg^8-vasopressin, oxytocin, or somatostatin. The antiserum was reconstituted with 25 ml distilled water and stored frozen in portions. A further 1:5 dilution with a dilution buffer (see below) was performed in conjunction with the assay. Human ^{125}I-ANF (1000 Ci/mmol, Milab, Malmö, Sweden) was reconstituted with distilled water and a dilution buffer containing 0.05 M sodium phosphate (pH 7.4), 0.05 M NaCl, 0.1% BSA, and 0.1% Triton X-100 to reach 5000 counts/min per 100 µl and frozen in small portions. Standard curves were constructed with synthetic alpha human ANF in phosphate/triton buffer over a concentration of 1.6 pg to 100 pg per tube. Samples consisted of a 100 µl aliquot of the unextracted plasma added to 100 µl of antiserum (triplicates). The mixture was vortexed and incubated at + 4°C overnight. The next day approximately 5000 counts/min (100 µl) of ^{125}I-ANF was added to each tube and incubated for an additional 24 h. On day 3, 250 µl of double antibody solid phase (DASP, dilution 1:20, Organon Teknika, Boxtel, The Netherlands) was added to each tube and the samples were vortexed at room temperature for 3 h and incubated for 3 days at + 4°C. On day 6, the samples were thoroughly vortexed and cold centrifuged (3600 rpm) for 30 min. The supernatant was eliminated and the samples were counted on a gamma counter. The assay IC_{50} was approximately 18 pg/tube. The recovery of ANF added to plasma was over 90%. The lower detection level was approximately 2 pg per tube. The interassay and intra-assay coefficients of variation were 10% and 4%, respectively, in the concentration range measured.

Values are expressed as mean ± SD, except for plasma Ir-ANF, for which the geometric mean and range are given. Paired observations of plasma Ir-ANF concentrations pre- and postdialysis and pre- and post-transplantation were compared using the Wilcoxon signed rank test. Linear regression analysis and stepwise multiple linear regression (Stat View SE + Graphics) were used to correlate plasma Ir-ANF values with post-transplant diuresis and dose of furosemide.

Results

Figure 1 shows the sequence of plasma Ir-ANF values for each patient and the geometric mean values. ANF levels were high prior to the last hemodialysis session and did not fall significantly during treatment in spite of a weight loss of 1.5 ± 1.1 kg. There was no significant correlation between weight loss and change in plasma Ir-ANF in the individual patient. In contrast, there was a correlation between pre- and postdialysis values ($r = 0.64$, $P = 0.03$).

The morning before transplantation, plasma Ir-ANF levels were the same as on the previous day, but that afternoon, after transplantation and volume expansion, a significant increase was recorded, from 143 to 391 pg/ml ($P = 0.02$). All grafts showed immediate onset with 24 h diuresis ranging from 3.7 to 17.8 l, the mean 6.6 ± 3.9. Seven patients who had a slow diuresis received furosemide. There was a mean increase in weight of 0.6 ± 1.5 kg until the next morning. Individual data are presented in Table 2. Stepwise multiple linear regression analysis showed that the post-transplant plasma Ir-ANF level was the strongest determinant of diuresis following transplantation ($r = 0.65$, F-test 7.41). The dose of furosemide on day 1 showed a negative correlation to diuresis, and when this was added to the post-transplant plasma Ir-ANF, the r-value was 0.87 and the F-test value 13.52 ($P = 0.002$). Graft function pretransplant did not seem to influence early diuresis (Tables 1, 2).

Table 1. Data on the living related kidney donors. GFR, Glomerular filtration rate; BSA, body surface area

Donor of patient number	Age (years)	GFR (ml/min per 1.73 m^2 BSA)	Urinary concentration capacity (mosmol/kg)
1	55	96	932
2	45	118	935
3	50	98	818
4	58	85	949
5	38	105	1030
6	34	126	808
7	41	78	1042
8	41	103	775
9	59	77	887
10	43	123	901
11	43	103	1036
12	45	126	856
13	67	91	1117
14	45	145	997

Table 2. Twenty-four hour diuresis following living related donor kidney transplantation in relation to plasma atrial natriuretic factor (ANF) levels 3–4 h post-transplant, furosemide dosage, and weight shift

Patient number	Diuresis (l/24 h)	Weight change (kg/24 h)	Furosemide (mg/24 h)	Plasma ANF post-transplant (pg/ml)
1	10.0	−2.7	0	730
2	4.5	+1.1	250	430
3	5.7	0	250	547
4	4.1	+3.1	0	110
5	17.8	0	0	1309
6	9.0	+1.8	0	322
7	4.7	+0.2	80	–
8	6.4	+1.5	0	360
9	5.0	+2.5	250	467
10	3.8	−0.2	375	907
11	4.1	−0.7	0	162
12	4.1	+1.3	0	–
13	10.0	−0.8	120	266
14	3.7	+1.2	125	243
Mean ± SD	6.6 ± 3.9	0.6 ± 1.5	–	488 ± 346
Geometric mean	5.9	–	–	391

Mean diuresis decreased to 3.3 ± 1.4 l on day 2 and to 3.0 ± 0.8 l on day 3 after transplantation in spite of administration of furosemide to more patients than on day 1 (10/14 vs 7/14) and with a maintained mean dosage (94 ± 95 mg vs 104 ± 128 mg). Natriuresis changed in parallel with diuresis from a geometric mean of 475 mmol/24 h on day 1 to 250 on day 3. By day 5 post-transplantation, 11 out of 14 patients had serum creatinine values less than 200 μmol/l and 9 had reached their true dry weight. Plasma Ir-ANF levels, however, remained in the same range as before transplantation (geometric mean 180 pg/ml). There was a trend towards a correlation between individual serum creatinine values and Ir-ANF (Fig. 2), but this was not statistically significant ($r = 0.37$). A significant reduction, to 63 pg/ml, was recorded 2–6 months post-transplant ($P = 0.03$, $n = 11$), and at follow-up after 1 year there was a further decline to 36 pg/ml ($P = 0.02$, $n = 9$). No correlation was seen between serum creatinine and Ir-ANF at 1 year post-transplant ($r = 0.03$) or between GFR and Ir-ANF in the long-term ($r = 0.19$).

Discussion

High concentrations of circulating ANF have previously been reported in uremic patients on chronic hemodialysis treatment [1, 9, 13–15, 18, 22, 23], in particular those patients with concomitant heart failure [10]. In general, studies comparing pre- and postdialysis values have demonstrated a moderate reduction in circulating ANF following dialysis. Some investigators have found a correlation between change in plasma ANF levels and change in weight [10, 13, 18], although this has not been a regular finding. Leunissen et al. [14], who recently reported that circulating ANF levels were more elevated in hypervolemic patients than in normovolemic patients, found that fluid removal confered reduced levels only on those who were hypervolemic. Plasma ANF values were, however, also increased in normovolemic patients. Thus, there is evidence to suggest that the uremic state might influence ANF levels through mechanisms other than acute fluid shifts. One such effect may be a reduced clearance of the hormone [14, 17, 22, 23]. This normally occurs via the neutral endopeptidase (NEP 24.11) located at the brush border of the tubular cells [2]. If this was the true explanation, a rapid normalization following successful renal transplantation with an undamaged graft could be anticipated. In fact, Woolf et al. [24], who followed up three transplanted patients for 8–23 days, reported a normalization of ANF values within 1 week in two of them. Raine et al. [16] have also presented data on nine recipients of cadaveric grafts followed up for 14 days. Those with functioning grafts (mean serum creatinine 210 μmol/l at the end of follow-up) reached an almost normal ANF concentration within this time while ANF values increased in three patients with nonfunctioning grafts. Using all observations for each patient, a significant correlation between serum creatinine and plasma ANF was obtained. In contrast, and in spite of better functioning grafts, normalization of plasma Ir-ANF was not seen until after several months in the present study. Furthermore, we found no clear correlation between renal function and plasma Ir-ANF at any time during follow-up. This might partly depend on the smaller range of renal function in our series. However, the fact that Ir-ANF levels on day 5 were also increased in patients with excellent graft function shows that renal function is not the major determinant of plasma ANF in this situation.

Intermittent overhydration is almost inevitably present in patients on hemodialysis and will affect cardiac function, even though clinical signs of cardiac failure may not appear as in the majority of these patients selected for renal transplantation. Our observation of a sustained increase in Ir-ANF levels following normalization of fluid balance and a near normalization of renal function might be explained as an effect of chronic stimulation caused by periods of fluid retention. This was suggested by Walker et al. [22], who demonstrated an exaggerated responsiveness

of ANF to saline infusion in patients with chronic renal failure. Their finding has been confirmed by Czekalski et al. [9], while data from water immersion studies in patients with acute renal failure indicate that their maximal capacity for ANF release is normal [12]. The chronic increase in plasma ANF in chronic renal failure makes the plasma ANF level a poor indicator of the patient's fluid state.

There is evidence to suggest that the renal response to increased circulating ANF is blunted in patients with advanced renal failure. No response was found to exogenous ANF [15, 21]. The intrinsic renal disease might be primarily responsible for this lack of effect, but evidence has also been put forward suggesting a down-regulation of ANF receptors as a result of the increased circulating levels of the peptide [15, 21]. When, in our study, normal kidneys were transplanted to uremic recipients, they were exposed to high plasma ANF concentrations that were further stimulated by the deliberate volume expansion. This type of regimen was adapted early in most transplant programs based on clinical experience. Onset of transplant function has been shown by Carlier et al. to depend on pulmonary arterial pressure [7], and the same group has provided evidence to suggest that ANF is involved as a mediator of the effect of hydration [8]. Our data lend further support to this view. Substitution of electrolyte losses may also increase the renal natriuretic effect of ANF that is abolished by sodium depletion [17].

Administration of synthetic ANF has been attempted following renal transplantation to promote initial graft function but with no positive effect [4, 20]. On the contrary, it seems that a drop in blood pressure following ANF administration in one set of experiments might have contributed to a high incidence of acute tubular necrosis [4]. These therapeutic failures might be due to factors such as dosage or timing of the ANF infusion. However, our results offer an alternative explanation to the lack of effect. Endogenous plasma ANF is increased in this situation and can readily be further stimulated by volume expansion. Normal kidneys transplanted under optimal conditions do respond to this endogenous ANF with natriuresis and diuresis. Damaged kidneys, on the other hand, may be unable to respond even to high levels of ANF, whether exogenous or endogenous, because the natriuretic effect depends on the renal perfusion pressure and several other factors [17].

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