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The effect of α-human atrial natriuretic peptide on the incidence of acute renal failure in cadaveric kidney transplantation

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Abstract. In previous studies in humans, mannitol (20%, 250 ml) has been shown to reduce the incidence of acute renal failure (ARF) after transplantation from 54% to 19%. In rats, atrial natriuretic peptide appears to prevent ischemia-induced ARF. We therefore decided to evaluate the effects of α-human atrial natriuretic peptide (α-h-ANP) both alone and combined with mannitol during transplantation in humans. First, we demonstrated that systemic α-h-ANP infusion during kidney transplantation was safe in dosages up to 0.08 μg/kg per minute. In these patients the calculated metabolic clearance rate of α-h-ANP was relatively low ranging from 0.68 to 1.80 l/min. In a second study of 11 renal graft recipients, no mannitol was used and α-h-ANP (0.05 μg/kg per minute) was infused into the donor kidney artery during transplantation for 46 ± 2 min, followed by IV administration for 71 ± 2 min. Our aim was to reduce the incidence of ARF. Nevertheless, ARF occurred immediately after surgery in four of the patients (36%) in this group and, as a result, mannitol was reintroduced. A third group of nine renal graft recipients received α-h-ANP (total dose 400 μg) as five IV injections within 90 min after transplantation. ARF occurred in four of these patients (44%). We conclude that α-h-ANP, administered according to the aforementioned protocols in such small groups of patients, does not seem to be of value in the prevention of ARF after transplantation.

Key words: α-Human atrial natriuretic peptide - Acute renal failure.

Acute renal failure (ARF) is a major postoperative complication of cadaveric kidney transplantation. This complication hampers the diagnosis of early rejection, increases the need for both diagnostic procedures and dialysis in the postoperative period, prolongs the recipient's stay in the hospital, and may even reduce graft survival [2]. The incidence of ARF after kidney transplantation varies from 30% to 60% [2, 3, 6] and can be reduced by maximal hydration [4]. However, this increases the risk of pulmonary edema and, consequently, the need for invasive monitoring of pulmonary artery pressure, and intermittent positive pressure breathing. We therefore looked for other effective, but clinically safer, regimens to reduce the risk of ARF after kidney transplantation.

In experimental animal studies, α-human atrial natriuretic peptide (α-h-ANP) has been found to prevent the development of ARF after artificial induction of renal ischemia [9, 12-14]. Given these results, we decided to study the effects of α-h-ANP on the incidence of ARF in cadaveric kidney transplantation in humans. Here, we present the results of α-h-ANP administration during transplantation in three groups of patients. First, a group of five patients was studied to evaluate whether α-h-ANP infusion was safe in anesthetized patients with chronic renal failure. Then, the incidence of ARF was studied after the administration of α-h-ANP alone and in combination with mannitol infusion in two groups of 11 and 9 patients, respectively. The latter variable was introduced since we had previously found that mannitol effectively reduces the incidence of ARF after cadaveric kidney transplantation [15].

Patients and methods

After approval of this study by the local ethics committee of our hospital, 25 transplant recipients (20 men, 5 women) gave their informed consent to participate in it. All suffered from end-stage

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renal failure and were treated either by intermittent hemodialysis (21 patients) or by peritoneal dialysis (4 patients) prior to transplantation. Transplant recipients were selected with the help of the Eurotransplant Foundation (Leiden, The Netherlands) on the basis of their HLA-DR, -B, and -A compatibility as well as their anti-HLA-antibody status. Mean (±SD) age, weight, and height of the patients were 47.4±12.6 years, 69.1±13.2 kg, and 172.5±9.9 cm, respectively.

All grafts were flushed and preserved in ice-cold Euro-Collins solution. Preoperative antibiotic prophylaxis, postoperative immunosuppressive therapy (with prednisolone and cyclosporin), and a moderate hydration policy during transplantation were standardized, as described previously [15]. Mannitol (20%, 250 ml), which was used only in the first and third study groups, was administered during the last 10 min before clamp removal.

In the first five renal transplant recipients (patients 1-5), we studied whether infusion of α-h-ANP was safe during anesthesia. Intravenous infusion of α-h-ANP was started in a very low dose just after clamp removal, and every 30 min the infusion rate was doubled (from 0.01 to 0.08 μg/kg per minute). If no contraindications existed, the highest dose of α-h-ANP was given for 90 min. Additional fluid was infused when systolic blood pressure fell below 120 mm Hg, or when heart rate increased above 130 bpm. If these measures did not have a sufficient effect, α-h-ANP infusion was halved or even stopped temporarily. Before and during α-h-ANP infusion, blood pressure and heart rate were monitored. Blood samples used to determine α-h-ANP concentrations [11] were drawn at the end of each α-h-ANP infusion rate period. Since plasma half-life of α-h-ANP is very short, also in renal failure [8], we assumed that during the highest α-h-ANP infusion rate, a steady state concentration of α-h-ANP would be reached after 90 min. Consequently, a metabolic clearance rate could be approximated by dividing the α-h-ANP-infusion rate by the difference between the steady state concentration and the basal level of plasma α-h-ANP.

After α-h-ANP infusion had appeared to be safe in anesthetized patients with renal failure, a second study was started in 11 renal transplant recipients (patients 6-16) in order to evaluate the influence of α-h-ANP on the incidence of ARF. During transplantation in these 11 recipients, no mannitol was given and, immediately after clamp removal, α-h-ANP (0.05 μg/kg per minute) was infused into the renal artery of the transplanted kidney via a 24-gauge catheter for a mean (±SE) time of 46.4±2.3 min (range 38-57 min). For surgical reasons, α-h-ANP was infused proximally into the iliac artery in two patients. To be sure of adequate intrarenal α-h-ANP concentrations in these two patients, the iliac artery was clamped just distally to the vascular anastomosis for the first 20 min of infusion. At the end of the operation, the intrarenal catheter was removed and α-h-ANP infusion was continued into a peripheral vein until a total duration of infusion of 2 h had been reached [mean (±SE) intravenous infusion time 71.4±2.4 min, range 57-82 min]. Blood pressure and heart rate were recorded as in the first study. Venous plasma α-h-ANP concentrations were measured before and at the end of the intrarenal and intravenous α-h-ANP infusions. At the end of the intrarenal infusion, we also took a renal vein blood sample. After transplantation, diuresis and endogenous creatinine clearance (ECC) were measured daily until the patient was discharged from the hospital. Since the nephrotoxic immunosuppressive therapy with cyclosporin was started 6 h after vessel clamp removal, diuresis was also registered separately during the first period of 6 h.

Because of the disappointing results of the second study, we evaluated the effect of intravenous bolus injections of α-h-ANP - this time combined with mannitol - in a third group of nine transplant recipients (patients 17-25). Immediately after clamp removal and 20 min later, 50 μg α-h-ANP was injected; in addition, three bolus injections of 100 μg α-h-ANP were given at 40, 60, and 90 min after clamp removal. Mannitol was given as in the first study. Plasma α-h-ANP concentrations were measured just before, and 5 min after, the third bolus injection of α-h-ANP. Diuresis and kidney function were monitored as in the second study group.

ARF was defined as a diuresis from the graft of less than 400 ml/24 h and/or the need for dialysis during the postoperative course, provided that the vascular anastomosis was patent as judged from radionuclide scans and that there were no signs of postrenal obstruction. Since all patients received a ureteric as well as a urethral catheter, urine produced by the graft could usually be distinguished from residual diuresis. If the urine production via the ureter was minimal, graft diuresis was calculated as the bladder diuresis minus the pretransplant diuresis. The diagnosis of acute rejection was made according to standard criteria. Graft biopsies were only taken when there were problems in distinguishing between acute rejection, ongoing tubular necrosis, and nephrotoxicity caused by cyclosporin.

All data were expressed as mean values ± standard error of the mean (SE). Statistical comparisons between groups were made with Student's *t*-test for unpaired observations. A *P*-value of 0.05 was considered as the level of statistical significance.

Results

Figure 1 shows the course of blood pressure and heart rate before and during the increasing α-h-ANP infusion rates in the first study. Additional fluid infusion was necessary in patients 1, 2, 3, and 5. Patient 3

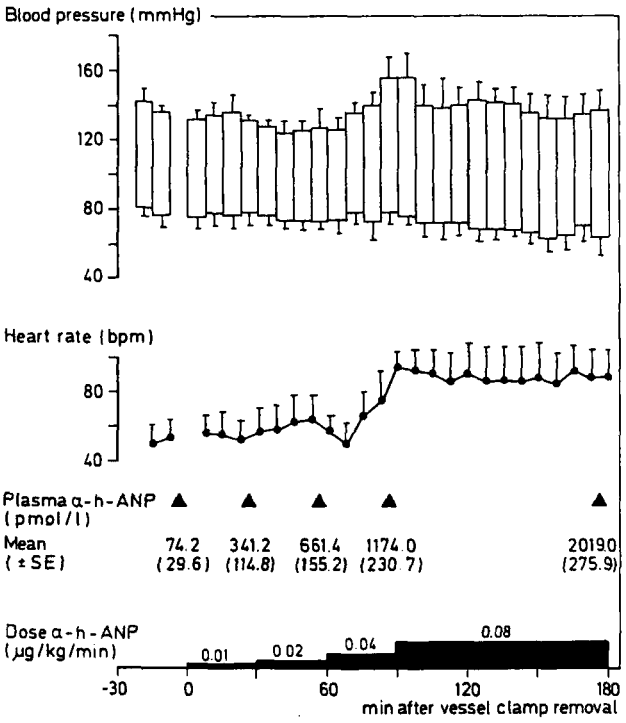

Fig. 1. The mean (±SE) course of blood pressure, heart rate, and plasma α-h-ANP concentration before and during intravenous infusion of α-h-ANP in increasing dosages from 0.01 to 0.08 μg/kg per minute. *Triangles* indicate blood sampling

also received packed erythrocytes because of excessive blood loss during surgery. The lowest blood pressure in this group was recorded in this patient as a result of hypovolemia (84/42 mm Hg). In all patients a prompt return to normal values occurred after additional fluid infusion. After about 90 min, the patients awakened from the anesthesia, and an increase in heart rate and blood pressure was observed at that time. Plasma concentrations of α-h-ANP are also shown in Fig. 1. The mean calculated metabolic clearance rate in this group was 1.00 ± 0.45 l/min (range 0.68–1.80 l/min).

Table 1 shows the individual values of diuresis after transplantation of the 11 recipients in the second group. According to our criteria, patients 6, 14, and 15 definitely suffered from ARF and so, for these patients, hemodialysis was necessary during the postoperative phase. In patient 10, ARF developed the 2nd day after graft vascularization. ARF lasted 3, 4, and 19 days in patients 10, 6, and 15, respectively, and up until nephrectomy of the transplanted kidney in patient 14 (37 days). The highest calculated ECC within the 1st month after transplantation are also presented in Table 1 and, for the patients with ARF (6, 10, 14, and 15), this maximum ECC was 73, 55, < 5, and 13 ml/min, respectively. For the other patients in this group, maximum ECC averaged 52.1 ± 5.1 ml/min (range 28–67 ml/min). The course of blood pressure and heart rate of the 11 recipients in this group was comparable to that in the first study group. The maximum percentage drop in systolic blood pressure varied from 5.0% to 26.8%, but no differences between the ARF and the non-ARF groups were observed (12.1% ± 7.3%; $n=4$ vs 14.4% ± 2.9%; $n=7$). The lowest recorded blood pressure in this group was 82/50 mm Hg (patient 6). In five patients (6, 9, 10, 11, and 15) additional fluid infusion was necessary, and in three of them (6, 9, and 10) α-h-ANP had to be stopped for 2.5, 6, and 7 min, respectively. During the α-h-ANP infusion in the second study group, the additional fluid infusion averaged 312 ± 244 ml in the ARF group and only 114 ± 77 ml in the non-ARF group ($P<0.05$). In the ARF group ($n=4$), plasma levels of α-h-ANP were 60 ± 12 pmol/l before and 419 ± 103 pmol/l (peripheral vein) and 1464 ± 607 pmol/l (renal vein) at the end of intrarenal infusion; they were 584 ± 191 pmol/l at the end of peripheral IV infusion. In the non-ARF group ($n=7$), these plasma α-h-ANP concentrations were comparable, that is, 82 ± 17 pmol/l, 480 ± 144 pmol/l, 1098 ± 387 pmol/l, and 706 ± 179 pmol/l, respectively. The mean time needed for construction of the vascular anastomosis did not differ between the ARF and the non-ARF groups; these were 36.3 ± 0.8 min (range 35–38 min) and 39.3 ± 3.3 min (range 28–55 min), respectively. As can be seen from Table 1, there were no relevant differences in the total ischemia time (TIT) between the group of patients with ARF and those without ARF (29.5 ± 4.9 h vs 27.4 ± 3.6 h). Aside from the grafts in patients 14 and 15, both of which came from the same donor, none of the grafts was subjected to any period of warm ischemia before transplantation.

Table 1. Individual values of post-transplant diuresis, total ischemia time (TIT), and maximum endogenous creatinine clearance (ECC) within the 1st month after transplantation of the 11 patients in the second study group

Patient number	Diuresis (ml)				TIT (h)	ECC (ml/min)
	0–6 h	Day 1[a]	Day 2	Day 3		
6[b]	0	312	220	400	43.9	73
7	170	792	1170	1795	36.9	63
8	1255	4176	1460	2440	8.4	63
9	182	936	1888	2082	23.6	50
10[b]	328	576	262	326	27.0	55
11	197	1104	1005	1580	27.2	47
12	325	1416	1460	2360	30.2	47
13	1100	4872	2735	725	33.7	67
14[b]	52	72	290	390	23.5	< 5
15[b]	93	336	65	15	23.5	13
16	744	2928	3190	950	31.6	28

[a] Day 1 was defined as the period from graft vascularization until 8:00 a.m. the following day. Values of diuresis presented for day 1 were linearly extrapolated to 24-h values
[b] Patients with acute renal failure (ARF) as defined in the text

Table 2. Individual values of post-transplant diuresis, total ischemia time (TIT), and maximum endogenous creatinine clearance (ECC) within the 1st month after transplantation of the nine patients in the third study group

Patient number	Diuresis (ml)				TIT (h)	ECC (ml/min)
	0–6 h	Day 1[a]	Day 2	Day 3		
17	1305	5749	3160	2100	33.9	43
18	460	1446	1840	3090	38.2	67
19[b]	0	0	60	183	37.1	16
20[b]	520	1346	700[c]	520[c]	35.3	< 5
21[b]	150[c]	887	1770	1735	36.0	57
22	?	1042	1910	2310	24.3	62
23[b]	?	288	156	180	44.7	< 5
24	605	1698	2250	1625	37.8	67
25	475	1254	2600	2000	35.0	41

[a] Day 1 as defined in Table 1
[b] Patients with acute renal failure (ARF) as defined in the text
[c] When corrected for residual pretransplant diuresis, graft diuresis was less than 400 ml/24 h

Table 2 shows the individual values of the diuresis after transplantation, as well as the TIT and the maximum ECC within the 1st month in the nine patients in the third study group. Three patients (19, 21, and 23) began to experience ARF immediately

after transplantation. In patient 20 diuresis decreased after the first 2 days to oliguric values. Hemodialysis was necessary in patients 19, 20, and 23. In two patients the renal grafts did not function within the first month. The mean calculated maximum ECC within the 1st month was 56.0 ± 5.8 ml/min in the rest of this group. All patients in the third group received additional fluid infusion to keep systolic blood pressure above 120 mm Hg. For three patients (18, 19, and 23), the total dose of α-h-ANP had to be reduced to 100, 350, and 300 μg, respectively, because of an α-h-ANP-related drop in blood pressure. The additional fluid infusion in this study group averaged 988 ± 471 ml in the ARF patients and 590 ± 274 ml in the non-ARF patients (not significant). The maximum percentage drop in systolic blood pressure in this group varied from 10.7% to 52.9%, but there were no differences between the ARF and the non-ARF groups (20.4% ± 4.9%; $n=4$ vs 29.4% ± 6.9%; $n=5$). Plasma α-h-ANP concentrations just before and after the third bolus injection were 288 ± 62 pmol/l and 1552 ± 376 pmol/l, respectively ($n=7$). In two patients no plasma α-h-ANP concentrations were available, and so a valid comparison between the ARF patients and non-ARF patients was not possible. The mean time needed for construction of the vascular anastomosis was 36.0 ± 6.9 min (range 16–46 min) in the four ARF patients and 33.4 ± 3.1 min (range 26–47 min) in the five non-ARF patients (not significant). The mean TIT was slightly higher in the patients with ARF than in those without ARF (38.3 ± 2.2 h vs 33.8 ± 2.5 h; not significant). The first warm ischemia time was 5 and 7 min for patients 18 and 19, respectively, and 0 min for the rest of this group.

Discussion

Continuous infusion of α-h-ANP in a dose of 0.1 μg/kg per minute induces only slight hemodynamic changes [5, 17]. However, during anesthesia, cardiovascular reflex reactivity may be reduced [16] and, consequently, a hypotensive response might be expected during α-h-ANP infusion and kidney transplantation. Moreover, the clearance of α-h-ANP may be reduced during chronic renal failure, as previously reported in anephric rats [8]. Therefore, we first carefully studied the response of blood pressure and heart rate to increasing infusion rates of α-h-ANP during kidney transplantation. We observed no adverse hemodynamic effects of dosages up to 0.08 μg/kg per minute. As illustrated in Fig. 1, mean blood pressure decreased slightly during α-h-ANP infusion.

As previously reported by others [10], we observed higher basal levels of α-h-ANP in our patients with chronic renal failure than in healthy volunteers, the former averaging 74.0 pmol/l ($n=16$) as compared to a range of 3–30 pmol/l in the latter [11]. In keeping with this, the calculated mean metabolic clearance rate in our patients was only 1.0 ± 0.5 l/min, whereas a higher clearance of α-h-ANP, averaging 6.0 ± 4.1 l/min, was recently found in healthy volunteers (unpublished observations).

In previous studies from our department in renal graft recipients, treated with the same antibiotic prophylaxis, immunosuppressive regimen and hydration policy as used in this study, mannitol infusion (20%, 250 ml) has been shown to decrease the incidence of ARF from 54% to 19% [15]. In the present study, a group of 11 patients received α-h-ANP intrarenally but no mannitol. According to our criteria, ischemic ARF occurred in 36% of these patients, while the blood pressure response to α-h-ANP in this group was similar to that in the non-ARF patients. As shown in Table 1, patient 16 developed ARF after starting cyclosporin infusion, suggesting that it may have been related to cyclosporin toxicity. In one rat model of ARF, ANP infusion time was 4 h [14], much longer than in our protocol. However, three other studies on the effect of ANP on ischemia-induced ARF in rats showed positive results despite shorter infusion times (20–60 min) [9, 12, 13]. Thus, it seems unlikely that the lack of efficacy of ANP in our study is related to the infusion time. The same holds for the dose of α-h-ANP that we used, as similar infusion rates (0.05 μg/kg body wt · per minute) have proven to be effective in preventing ARF in at least one study [12]. If we had used higher doses of α-h-ANP in our patients, monitoring of central hemodynamic parameters would have been necessary. In that case, such a protocol would offer no advantages when compared to protocols with maximal hydration, which have proved to be effective in reducing ARF incidence in humans [4]. Thus, despite the small number of patients in this study, and because our results with mannitol are similar or even better than those with α-h-ANP and the procedure is much less cumbersome, we feel that mannitol infusion is preferable to intrarenal infusion of α-h-ANP for reducing the incidence of ARF.

In the experimental literature there is some evidence that bolus injections of ANP induce dose-dependent reductions in renal vascular resistance, whereas continuous infusion exerts an opposite response [7]. After denervation of the kidney, a fall in renal blood flow as seen during continuous ANP-infusion in animals with normally innervated kidneys was not observed, but still no decrease in renal

vascular resistance, as observed after bolus injections, occurred. We therefore included bolus injections of α-h-ANP in our last study group and combined this with mannitol to achieve a further reduction in ARF incidence. The more than fivefold increase in plasma α-h-ANP concentration from just before to just after the third bolus injection shows that our schedule of five injections induced intermittently high α-h-ANP concentrations. Nonetheless, in this third study group, four out of the nine patients (19, 20, 21, and 23) developed ARF. In patient 20, it may have been related to cyclosporin toxicity. Thus, according to the present results, neither continuous intrarenal infusion of α-h-ANP nor intravenous α-h-ANP bolus injections seems to be of value in the prevention of ARF after cadaveric kidney transplantation. We have no clear explanation for the discrepancy between the beneficial effects of ANP on ischemia-induced ARF in rats [9, 12-14] and our current results. Differences in intrarenal hemodynamic effects of ANP between species may play a role [1]. Moreover, the intrarenal hemodynamic effects of α-h-ANP may be altered by the denervated state of the transplanted kidney.

We conclude that the administration of α-h-ANP in dosages up to 0.08 μg/kg per minute is a safe procedure during kidney transplantation in humans. In this particular group of patients, basal plasma α-h-ANP concentrations were relatively high, at least partly due to a drop in metabolic clearance rate. We further conclude that α-h-ANP, administered according to the aforementioned protocols, does not seem to be of value in the prevention of ARF after cadaveric kidney transplantation. More studies are needed to evaluate the effects of α-h-ANP on ischemia-induced ARF from other causes.

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