Atrial Natriuretic Factor Does Not Improve the Outcome of Cadaveric Renal Transplantation[1,2]

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ABSTRACT

Atrial natriuretic factor (ANF) ameliorates renal damage in animal models of acute ischemic renal failure. Consequently, ANF could blunt acute tubular necrosis related to ischemia that occurs frequently in cadaveric renal transplants. Ten pairs of cadaveric kidneys were transplanted into 20 recipients. Paired recipients received either alpha-human ANF (hANF) or vehicle alone in a prospective, double-blind protocol. Upon revascularization of the allograft, either hANF or vehicle was administered intravenously as a 50-μg bolus, followed by a 4-h infusion (0.1 μg/kg/min). Glomerular filtration rate ((^{125}I)iothalamate clearance) was measured between 4 and 7 days posttransplant and again between 14 and 21 days posttransplant. Serum creatinine was measured daily when patients were in the hospital, then twice weekly as patients were examined in the outpatient clinic. Between the groups, there was no significant difference in age of the recipients or donors, cold ischemia time, or histocompatibility leukocyte antigen match. Infusion of hANF had no adverse effects. When subjects receiving hANF were compared with those treated with vehicle alone, there were no significant differences in serum creatinine or glomerular filtration rate. Three hANF and four vehicle recipients required dialysis postoperatively. At 1 month posttransplant, 19 of 20 patients had functioning allografts; an allograft from one hANF recipient never functioned. It was concluded that hANF, when given by the protocol of this study, had no beneficial effect on the outcome of cadaveric renal transplantation in humans.

Key Words: *Renal transplant, acute tubular necrosis, ischemia, atrial natriuretic factor, human*

Atrial natriuretic factor (ANF) is a 28-amino-acid peptide hormone which induces a natriuresis and diuresis as part of the physiologic response to an increase in intravascular volume (1–3). Pharmacologic concentrations of ANF cause a marked increase in glomerular filtration rate (GFR) (1–3). Because of this property, several investigators have tested whether ANF could ameliorate ischemic renal injury in animal models, including renal artery clamping or norepinephrine infusion into rats. These studies showed that ANF, given immediately after ischemic renal injury, significantly improves creatinine clearance, reduces serum creatinine, and reduces the amount of histologic acute tubular necrosis (4–13).

The beneficial effect of ANF in ameliorating ischemic acute renal failure raises the possibility that ANF might also be useful in preventing ischemic damage after cadaveric renal transplantation of humans. Acute renal failure arises because donor kidneys are ischemic after being harvested until transplantation into the recipient. Prolonged "cold storage" of cadaveric kidneys has been associated with an increased incidence of ischemic damage, delayed graft function, and poor graft survival (14–16). Fortunately, ANF has little toxicity, has a half-life in plasma of 2 to 3 min (1), even in subjects with end-stage renal disease (17), and has been administered safely to both normal volunteers (18–21) and patients with renal insufficiency (17,22).

Two recent articles have suggested the need for a

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[3] J.M. Sands dedicates this report to the memory of Dr. Chester Landy.

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controlled clinical trial of ANF in renal transplants (23,24). To date, no controlled studies have been performed. Consequently, we used a randomized, double-blinded, placebo-controlled study to determine whether ANF, administered immediately posttransplant, would benefit renal function in cadaveric renal allografts in humans. An improvement in renal function would likely be associated with a decrease in postoperative complications, dialysis dependence, and a briefer hospitalization, and might increase the available pool of donor organs.

METHODS

Patients

Participants in the Emory Transplant Program were selected prospectively from patients with ESRD awaiting cadaveric renal transplantation. The study was approved by the Emory University Human Investigations Committee and the U.S. Food and Drug Administration; voluntary informed consent was obtained from all participants before entry into the study.

ANF Protocol

Paired kidneys from 10 consecutive cadaveric donors were transplanted into 20 recipients. The recipients from each pair were assigned to either the experimental or control group; the experimental group received alpha-human ANF (hANF, 28 amino acids; Peninsula Laboratories, Belmont, CA), and the control group received vehicle (0.9% saline) alone. The assignment of subjects was performed in a randomized, double-blinded manner. At the time of revascularization of the allograft, each recipient received a 50-μg bolus of hANF intravenously or an equivalent amount of vehicle, followed by a 4-h infusion of hANF (0.1 μg/kg/min) or vehicle. This dose of hANF was chosen because it is sufficient to cause a natriuresis and diuresis without producing a major change in mean arterial pressure in normal humans (20,21). In addition to hANF or vehicle, all patients received a standard regimen of extracellular volume expansion with saline, albumin, and mannitol, plus furosemide at the time of allograft revascularization.

Measurement of Allograft Function

To assess function of the transplanted kidney, serum creatinine was measured daily until the patient was discharged from the hospital, then twice a week in clinic for the first month posttransplant. GFR was measured by [^{125}I]iothalamate clearance between the 4th and 7th day postoperatively and again between the 14th and 21st day postoperatively. [^{125}I]iothalamate clearance was not measured in one patient (see Table 2); a 24-h creatinine clearance was used to estimate GFR. We did not measure the GFR of those patients requiring dialysis in the immediate posttransplant period. Instead, we assigned these patients a value of 0 GFR.

Immunosuppressive Therapy

All patients received ABO-compatible, lymphocytotoxicity cross-match-negative, cadaveric allografts. Induction therapy included Minnesota antilymphocyte globulin (15 mg/kg for 5 to 14 days), azathioprine (1.0 to 2.0 mg/kg/day), and methylprednisolone (325 mg tapered to 30 mg/day by postoperative day 6). Cyclosporine (10 mg/kg/day) was introduced when the serum creatinine fell below 3.0 mg/dL, and Minnesota antilymphocyte globulin was discontinued when cyclosporine blood levels reached 300 to 400 ng/dL when measured by whole blood, monoclonal radioimmunoassay. Maintenance immunosuppression consisted of cyclosporine, azathioprine, and prednisone.

Statistics

All data are presented as mean ±SE. A paired Student's t test was used for comparisons between groups. A nonparametric rank-sum test was used where indicated in the Results. The criterion for statistical significance was $P < 0.05$.

RESULTS

Patient Characteristics

There was no significant difference in recipient age or sex, cold ischemia time, or histocompatibility leukocyte antigen match in the patients who received hANF and those who received vehicle (Table 1).

Effect of hANF

All ANF recipients had a transient decrease in systolic blood pressure after the hANF bolus (mean, 10.0 ± 1.2%, $P < 0.001$). Blood pressure returned to prebolus values within 5 min. Vehicle recipients had no significant change in systolic blood pressure after the bolus (3.5 ± 2.5%). There was no significant decrease in central venous pressure in either group (ANF, 6.3 ± 3.3%; vehicle, 0.0 ± 0.0%). No patient became hypotensive, and hANF administration had no other adverse effects. At 24 h postoperatively, there was no difference in arterial blood pressure (ANF, 141 ± 7/79 ± 3; vehicle, 141 ± 8/83 ± 5) or

TABLE 1. Patient characteristics[a]

	Case	Age	Sex	Donor Age	Ischemic Time	HLA Match	Hypertension	Cause ESRD
ANF-treated patients	1	22	F	21	23.7	1A, 2B, 1DR	Yes	Reflux nephropathy
	2	34	M	54	34.2	0A, 0B, 1DR	Yes	Hypertension
	3	19	M	20	31.0	0A, 1B, 1DR	No	Chronic GN
	4	64	M	24	33.3	0A, 1B, 1DR	No	MPGN
	5	53	F	50	18.0	0A, 0B, 1DR	Yes	Diabetes
	6	53	M	26	18.1	1A, 0B, 1DR	Yes	Hypertension
	7	54	F	33	27.5	1A, 1B, 0DR	Yes	Hypertension
	8	61	M	54	36.0	0A, 0B, 1DR	Yes	Hypertension
	9[b]	21	M	16	23.9	2A, 1B, 1DR	Yes	IgA nephropathy
	10	60	M	25	12.6	0A, 1B, 1DR	Yes	Chronic GN
	Mean	44.1 ± 5.7	7M, 3F	32.3 ± 4.7	25.8 ± 2.5	2.1 ± 0.3		
Vehicle-treated patients	1[b]	21	F	21	28.3	1A, 2B, 1DR	Yes	FSGN
	2	50	F	54	28.9	0A, 1B, 1DR	Yes	FSGN
	3	57	M	20	27.3	1A, 0B, 1DR	Yes	Adult polycystic
	4	29	F	24	33.0	0A, 0B, 2DR	No	SLE
	5	58	M	50	14.1	0A, 1B, 1DR	No	Membranous GN
	6	47	F	26	31.3	0A, 1B, 0DR	Yes	Chronic GN
	7	23	F	33	14.3	0A, 0B, 1DR	Yes	Interstitial nephritis
	8	50	M	54	34.8	0A, 1B, 0DR	Yes	Diabetes
	9	47	F	16	24.2	1A, 1B, 1DR	No	Diabetes
	10	31	M	25	13.2	1A, 0B, 1DR	Yes	Diabetes
	Mean	41.3 ± 4.4	4M, 6F	32.3 ± 4.7	24.9 ± 2.6	2.0 ± 0.3		

[a] Abbreviations are as follows: GN, glomerular nephritis; MPGN, membranoproliferative glomerulonephritis; FSGN, focal segmental glomerulonephritis; SLE, systemic lupus erythematosus.

[b] Retransplant.

central venous pressure (ANF, 10.4 ± 0.8; vehicle, 11.7 ± 1.8). Total urine output in the first 24 h postoperatively was similar in both groups (ANF, 3.6 ± 1.2 liters; vehicle, 3.7 ± 1.2 liters).

Allograft Function

Posttransplant dialysis was required for three patients receiving hANF and four patients receiving vehicle (Table 2). There was no significant difference in allograft function between hANF recipients and vehicle recipients. GFR at 4 to 7 days posttransplant (hANF, 33.9 ± 9.2; vehicle, 27.9 ± 9.5) or at 14 to 21 days posttransplant (hANF, 51.0 ± 8.9; vehicle, 50.8 ± 7.1) were not different. Serum creatinine at 3 days (hANF, 7.4 ± 1.1; vehicle, 6.9 ± 1.4), 1 week (hANF, 4.7 ± 1.3; vehicle, 5.2 ± 1.4), or 1 month posttransplant (hANF, 2.9 ± 1.0; vehicle, 1.9 ± 0.8) were not different. It should be emphasized that serum creatinine values for all patients, including those being dialyzed, were included. Because of this factor and the fact that creatinine values in several patients were changing, creatinine did not approximate GFR accurately. Regardless, outcome was the same if data were analyzed by Student's *t* test or by the nonparametric, rank-sum test or when patients not requiring dialysis were considered separately. Allograft function was present in all patients at 1 month posttransplant, except for one ANF recipient whose allograft never functioned and was removed. Analysis of these data also revealed no significant difference in allograft function between the two groups of patients.

We also used the GFR results to estimate how many patients would have to be studied to determine that the small difference in GFR we detected would be statistically significant assuming the same degree of variability would apply for a larger group of patients. It can be calculated that 97 subjects would be required to determine that hANF statistically improved GFR after 4 to 7 days.

DISCUSSION

In this prospective, randomized, placebo-controlled, double-blinded study of 10 pairs of patients, we were unable to demonstrate any beneficial effect of hANF, given by our protocol. The lack of a difference in preserving the function of cadaveric renal transplants could not be linked to variability in age of the patients or donors, cold ischemia time, or HLA match. At the dosage we used, hANF had no adverse effects on the patients or, as far as we could detect, allograft function. Our result is disappointing consid-

TABLE 2. Measurements of renal function[a]

	Case	Creatinine (3 days)	GFR (4 to 7 days)	Creatinine (1 wk)	GFR (2 to 3 wk)	Creatinine (1 month)
ANF-treated patients	1	1.9	51.6	1.3	54.8	2.1
	2	11.3	Dialysis	11.3	Dialysis	11.5
	3	3.7	76.9	1.4	66.1	1.2
	4	9.3	8.9	4.9	Refused[b]	1.4
	5	8.3	Dialysis	9.1	ND[c]	2.3
	6	6.0	62.5	2.7	64.8	2.1
	7	8.2	42.9	3.3	47.1	2.3
	8	12.6	Dialysis	9.6	ND	2.6
	9	4.1	46.4	1.7	64.8	2.1
	10	8.2	50.0	1.7	59.3	1.6
	Mean	7.4	33.9	4.7	51.0	2.9
	SE	1.1	9.2	1.3	8.9	1.0
	N	10	10	10	7	10
Vehicle-treated patients	1	1.4	69.6	2.0	46.7	1.3
	2	10.6	Dialysis	9.5	ND	3.2
	3	6.1	47.2	1.7	74.3	1.3
	4	5.8	23.2	2.6	30.6	2.0
	5	14.2	Dialysis	11.9	ND	3.2
	6	9.7	Dialysis	9.3	ND	1.5
	7	10.0	Dialysis	9.5	ND	1.9
	8	8.5	13.9	3.6	47.6	1.8
	9	1.3	52.0[d]	0.8	54.7	1.1
	10	1.8	73.3	1.3	Refused[b]	1.6
	Mean	6.9	27.9	5.2	50.8	1.9
	SE	1.4	9.5	1.4	7.1	0.2
	N	10	10	10	5	10

[a] Creatinine, serum creatinine measured in milligrams per deciliter. GFR was measured by (^{125}I)iothalalmate clearance (in milliliters per minute). Patients on dialysis were assigned a GFR of 0 mL/min for statistical purposes.

[b] Patient refused follow-up GFR study.

[c] ND, not done (follow-up GFR were obtained only in those patients who had an initial GFR study).

[d] This GFR was determined by creatinine clearance as patient was unable to undergo an (^{125}I)iothalamate study. Follow-up GFR was determined by (^{125}I)iothalamate clearance.

ering the potential for a beneficial effect which has been demonstrated in animal studies (4–13).

Studies of ischemic acute renal failure in animals have examined different combinations of ANF boluses and infusions, but ANF was not administered for more than 4 h in any one study (4–13). We based our protocol on these results and administered hANF as a bolus, followed by a 4-h infusion. We used a dose (0.1 μg/kg/min) which is more than sufficient to produce a physiologic effect in normal human kidneys but would not be expected to reduce mean arterial blood pressure by greater than 10% (20,21). In fact, this dose is 10-fold higher than the amount that was shown to decrease central venous pressure in normal subjects (19) and is four times greater than the 0.025 μg of hANF/kg/min dose which was shown to produce natriuresis and diuresis regularly in normal subjects (19). We decided not to use a higher dose because of potential hypotensive effects in postoperative patients; a dose of 100-μg bolus followed by an infusion of 20 μg/min reduced mean arterial pressure by 30% (25). Our hANF recipients experienced an average decrease in systolic blood pressure of 10% after the hANF bolus, consistent with the dose of hANF administered. Still, it remains possible that a longer infusion or a higher dose could prove beneficial in preserving renal function.

These results do not mean that ANF plays no role in preserving renal function after transplantation because extracellular volume expansion is a major stimulus for endogenous ANF secretion (1–3). All patients received conventional therapy with intravenous saline, mannitol, and high-dose furosemide intraoperatively, and it is possible that the beneficial effect of volume expansion on cadaveric renal transplants is due in part to stimulation of endogenous ANF secretion (22,26). One study found that both ANF and mannitol were needed to protect against ischemic injury in isolated perfused kidneys (7). Our results suggest that hANF alone would not be superior to conventional extracellular volume expansion.

Minnesota antilymphocyte globulin was used

rather than cyclosporine as part of the initial immunosuppression. Consequently, we did not address the possibility that ANF would be beneficial in ameliorating vasoconstriction occurring when cyclosporine is administered as part of initial immunosuppressive therapy (16,27,28). Available evidence suggests that even with cyclosporine, ANF is not beneficial. In one study, ANF was given for 90 min to 20 patients at the time of allograft revascularization (29). Other therapies were not standardized (some subjects were given mannitol), and renal function was assessed only by serum creatinine and compared with results from historical controls. Those investigators concluded that ANF was not beneficial, as 40% of the patients had clinical evidence of ischemic acute renal failure postoperatively (29). Another study failed to find a beneficial effect of ANF in six patients with established oligoanuric renal failure after cadaveric renal transplantation (25). Two patients had a diuresis, but four had no response to ANF; a control group was not included in this study, and changes in renal clearance were not evaluated.

In summary, we did not find any beneficial effect of hANF over conventional therapy in a prospective, randomized, placebo-controlled, double-blinded study of hANF in cadaveric renal transplant recipients. No adverse effects were noted at the dose used.

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