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A prospective randomised study of the effect of nicardipine on ischaemic renal injury in renal allografts

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Abstract In a prospective double-blind trial, 127 kidneys were randomised to receive Eurocollins ($n = 65$) or Eurocollins plus nicardipine ($n = 62$) as a second flush solution at the time of organ retrieval. Delayed graft function occurred in 18 of 65 control kidneys (28 %) and in 20 of 62 nicardipine kidneys (32 %; $P = 0.7$, Fischer's exact test). The mean (SD) serum creatinine at 6 weeks was 197 (138) μmol/l in the Eurocollins group and 195 (159) μmol/l in the nicardipine group ($P = 0.95$). Eighteen recipients (28 %) in the controlled Eurocollins group experienced a rejection episode in the first 6 weeks post-transplant compared to 17 (27 %) in the nicardipine group (χ^2 with Yates' correction = 0.027; $P = > 0.95$). In this study, the addition of nicardipine to the kidney perfusion fluid did not have a beneficial effect on kidney function following transplantation.

Key words Nicardipine, kidney transplantation, preservation · Kidney transplantation, nicardipine · Preservation, nicardipine

Introduction

Delayed graft function (DGF) is a common occurrence in cadaveric renal transplantation, occurring in approximately 40 % of recipients. Patients with DGF require post operative dialysis and present more management problems than those with initial function. Acute allograft rejection is more difficult to diagnose during DGF and there is a higher requirement for invasive investigations such as needle core biopsies. In addition, there are a number of studies suggesting that DGF is associated with poorer 1-year graft survival results [1–3, 16, 19].

Extensive research using animal models suggests that calcium channel blockers limit ischaemic damage and improve renal function following periods of warm and cold ischaemia [10, 12, 14, 17, 22, 23]. Addition of calcium channel blockers to the perfusion fluid at organ retrieval has also been shown to reduce DGF in randomised human studies [4, 15, 18, 20]. In addition, DGF was reduced by verapamil pre-treatment in an animal model mimicking unstable donors [1].

The aims of this study were to determine the effects of the addition of the new calcium channel blocker nicardipine to the kidney perfusion fluid. The main end points of the study were the number of cases of DGF, the renal function at 6 weeks post-transplant and the incidence of acute rejection in the first 6 weeks.

Materials and methods

A consecutive series of 65 heart-beating cadaveric organ donors were entered into the study. At the retrieval operation the kidneys were rapidly cooled using an in situ perfusion technique. The initial perfusion fluid varied according to the type of organs being procured, with University of Wisconsin solution being used during multi-organ retrieval and hyperosmolar citrate during kidney only retrievals. After initial perfusion both kidneys were removed en bloc and then separated. Each donor kidney was then randomised to receive an extracorporeal second flush with either 500 ml Euro-

collins solution or 500 ml Eurocollins solution containing 2 mg nicardipine. The kidneys were then triple-wrapped for dispatch in bags containing 100 ml of the second flush solution and stored on ice for transport to their respective recipients. The study was double-blind, the randomisation procedure being carried out by a transplant coordinator who was not involved in the subsequent clinical assessment of outcome. Donor kidneys were allocated to recipients in the Leicester area or offered nationally via the UK-TSS. The main investigator (MLN) liaised with all appropriate transplant units for data collection.

Following transplantation serum creatinine, blood cyclosporin levels, 24-h urine volumes, blood pressure and heart rate were recorded on a daily basis. The number of days to produce both a spontaneous fall in serum creatinine of 50 μmol in 24 h and 1000 ml of urine in a 24-h period were noted. For patients with delayed function the number of haemodialyses and the number of days of continuous ambulatory peritoneal dialysis (CAPD) were noted. All patients were followed up 6 weeks after transplantation for the assessment of renal function, number of rejection episodes and the incidence of, and reasons for, graft failure. Renal transplant recipients in Leicester had their 6-week post-transplant glomerular filtration rate (GRF) measured by an isotopic method.

The two treatment regimens were compared using a chi-squared test on the binary response variable recipient needed dialysis or did not need dialysis. A sign test was used to test the treatment effect taking into account the paired nature of the data. For each pair if dialysis was required by a recipient in the Eurocollins group but not by a recipient in the nicardipine group then a positive result was recorded; a negative result was recorded if dialysis was required by a recipient in the nicardipine group but not by a recipient in the Eurocollins group. A tie was recorded if both recipients in the pair had the same results. The duration of dialysis and the time to a spontaneous fall in the serum creatinine of 50 μmol/l were compared in the two treatment groups using Kaplan-Meier estimates and the log-rank test. Serum creatinine and GFR measurements at 6 weeks were compared between the two treatment groups using analysis of variance. Recipients who died, had the kidney removed or had a non-functioning kidney were excluded from this analysis. The incidence of DGF and rejection episodes during the first 6 weeks were compared between the nicardipine and control groups using Fisher's exact test. All statistical analyses were two-tailed and carried out at the 5 % level of significance.

Results

There were a total of 65 donors in the study making a possible 130 kidneys available. Three of these kidneys were never transplanted and, of the remaining 127 kidneys 65 were randomised to the control Eurocollins group and 62 to the nicardipine group. In the Eurocollins group 34 left kidneys and 31 right kidneys were used compared with 30 left kidneys and 32 right kidneys for the nicardipine group.

Donor details

Donor age ranged from 10 to 75 years with a median of 42 years. There were 38 male donors (58 %) and 27 female donors (42 %). The mean (SD) urine output in the 24 h prior to organ retrieval was 3064 (1272) ml with a range of 532–6258 ml. The mean (SD) serum creatinine was 92 (33) μmol/l with a range of 37–209 μmol/l ($n = 60$).

Recipient demography

The details of the 127 recipients are summarised in Table 1. The control and study groups were well matched for age, sex, weight, blood pressure, original disease and pre-transplant cytotoxic antibodies. There were significantly more Asian patients ($\chi^2 = 7.7$, $df = 2$, $P < 0.05$) and a higher proportion of patients being dialysed by CAPD alone ($\chi^2 = 8.42$, $df = 3$, $P < 0.02$) in the control group.

Risk factors

A number of potential risk factors for patients undergoing renal transplantation were identified and these have been summarised in Table 2. In the Eurocollins group 35 recipients (55 %) were transplanted at Leicester and 30 recipients (45 %) were transplanted elsewhere. The corresponding numbers for the nicardipine group were 26 recipients in Leicester (42 %) and 36 elsewhere (58 %). Comparison of the risk factors for the two treatment groups indicated no significant disparity between them.

Delayed graft function

After transplantation of the kidney, 18 recipients (28 %) in the Eurocollins control group and 20 (32 %) in the nicardipine group had DGF as defined by the requirement for dialysis in the 1st post-transplant week. There was no significant difference in the proportion of recipients requiring dialysis between the two groups ($P > 0.7$, Fisher's exact test). The sign test for the difference in the two groups taking into account the paired nature of the data gave a P value of 0.678.

The median (95 % confidence interval) duration of dialysis in the Eurocollins control group was 12 (9.0–18.8) days and in the nicardipine group was 13 (9.4–22.6) days (Mann-Whitney U statistic 171.5, $P = 0.815$). For the recipients receiving dialysis, the log-rank test gave a value of $P = 0.51$, indicating no difference between the two groups for the duration of dialysis. This is shown in Fig. 1, where the probability of continuing in the study with no further need for dialysis has been plotted.

The time to a spontaneous fall in serum creatinine of more than 50 μmol/l ranged from 0 to 36 days in the Eurocollins group and form 0 to 49 days in the nicardipine group; the median was 0 days in both groups. There was no significant difference between the time to fall in

Table 1 Summary of recipient demography

		Control group (Eurocollins) ($n = 65$)	Test group (Eurocollins nicardipine) ($n = 62$)
Age (years) Median (range)		42 (5–71)	43 (5–68)
Sex[a]			
Male		41 (64 %)	36 (59 %)
Female		23 (36 %)	25 (41 %)
Race[a]			
Caucasian		55 (86 %)	59 (97 %)
Asian		9 (14 %)	1 (1.5 %)
Black		0	1 (1.5 %)
Weight (kg)			
Mean (SD)		64.6 (14.1)	62.4 (16.0)
Range		18–93.8	15–98
Systolic BP (mm Hg)			
Mean (SD)		150 (27)	147 (30)
Range		100–220	96–240
Diastolic BP (mm Hg)			
Mean (SD)		76 (11)	75 (12)
Range		60–116	60–110
Total number of recipients		65	62
Type of dialysis			
Haemodialysis only		21 (34 %)	28 (47 %)
CAPD only		36 (58 %)	20 (33 %)
Haemodialysis + CAPD		5 (8 %)	12 (20 %)
Unknown		3	2
Original disease			
Chronic		17	12
Pyelonephritis		5	6
Nephrosclerosis		0	4
Polycystic		10	10
Diabetes		6	3
Other		27	27
Pre-transplant cytotoxins against panel			
Highest serum	00–50	62	59
	51–75	1	0
	> 75	0	1
	Unknown	2	2
	Maximum	67	97
Latest serum	00–50	62	60
	51–75	1	0
	> 75	0	0
	Unknown	2	2
	Maximum	63	35

[a] One recorded in each group

Table 2 Summary of risk factors

	Control group (Eurocollins) ($n = 65$)	Test group (Eurocollins nicardipine) ($n = 62$)
Total number of recipients	65	62
Age		
< 40 years	25 (39 %)	26 (43 %)
≥ 40 years	39 (61 %)	35 (57 %)
Unknown	1	1
Transplant centre		
Leicester	35 (54 %)	26 (42 %)
Other	30 (46 %)	36 (58 %)
Transplant status		
First	57 (88 %)	54 (87 %)
Second/third	8 (12 %)	8 (13 %)
HLA mismatch		
0	2	2
1	7	13
2	15	17
3	23	14
4	14	10
5	3	5
6	0	0
Unknown	1	1
Median	3	2
Crossmatch test		
Positive	4 (6 %)	2 (3 %)
Negative	61 (94 %)	60 (97 %)
Immunosuppression		
Received CyA	65	62
Did not receive CyA	0	0

serum creatinine between the two groups (log-rank test, $P = 0.58$; Fig. 2).

Concomittant pre- and post-operative calcium antagonist therapy

None of the donors received calcium channel blockers in the 48 h prior to organ donation. Nifedipine was being taken in the pre-operative period by 36 of the 127 recipients. The incidence of DGF in these patients was 10 out of 36 (28 %) in comparison with 28 out of 91 patients (31 %) not receiving pre-operative calcium channel blockers ($P = 0.83$, Fisher's exact test). Post operatively a total of 63 recipients received calcium channel blocker therapy. Nifedipine was taken by 31 recipients in the Eurocollins control group and 31 patients in the nicardipine group; a single patient n the Eurocollings control group was administered diltiazem. The incidence of DGF in patients receiving post-operative calcium channel blockers was 14 out of 63 patients (22 %) in comparison with 24 out of 64 patients (38 %) not receiving calcium channel blockers ($P = 0.08$, Fisher's exact test). In the 32 Eurocollins control patients receiving post-operative calcium channel blockers, the incidence of DGF was 6 out of 32 patients (19 %) in comparison with 12 out of 33 (36 %) of Eurocollins control patients not receiving post operative calcium channel blockers ($P = 0.17$, Fisher's exact test). In the nicardipine group 8 out of 31 patients (26 %) receiving post operative calcium channel blockers had DGF in comparison with 12 out of 31 patients (39 %) who did not receive post operative calcium channel blockers ($P = 0.42$, Fisher's exact

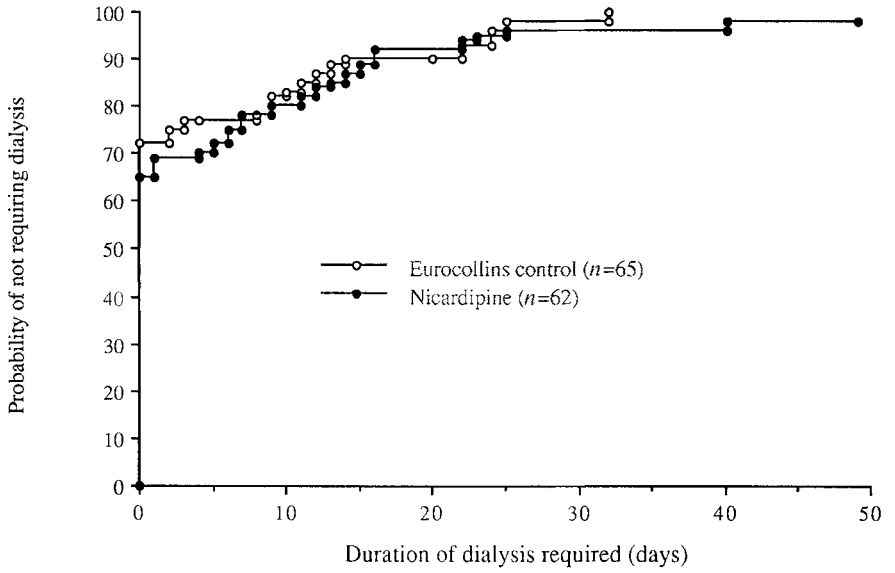

Fig. 1 Probability of continuing in the study with no further need for dialysis. $P = 0.51$

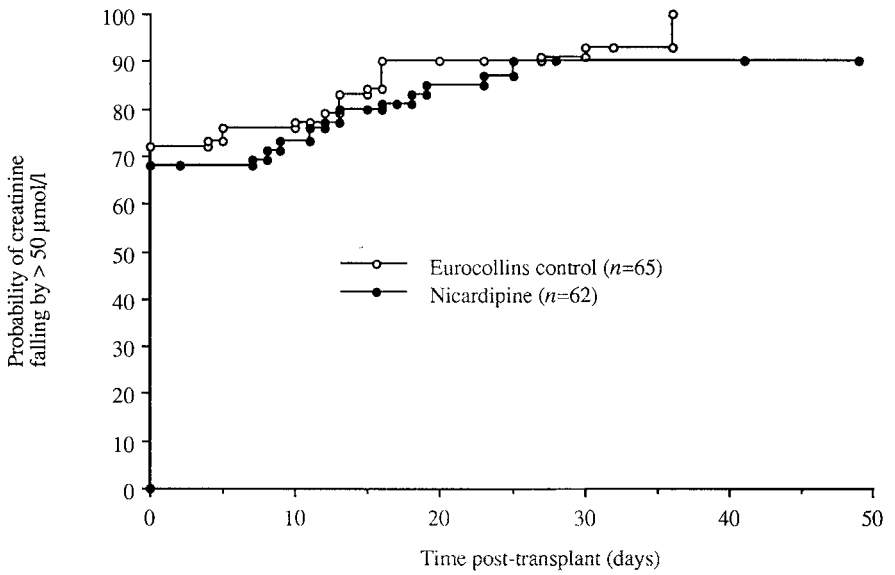

Fig. 2 Probability of reduction in serum creatinine of 50 μmol/l. $P = 0.58$

test). The incidence of primary non-function (patients never achieving independence from dialysis post-transplantation) was 2 out of 62 patients (3.2 %) in the nicardipine group compared with 2 out of 65 patients (3.1 %) in the Eurocollins control group ($P = 1.0$, Fisher's exact test).

Six-week follow-up

In the Eurocollins group ($n = 48$), the mean (SD) serum creatinine at 6 weeks was 197 (138) μmol/l with a range of 38–795 μmol/l. The mean (SD) serum creatinine in the nicardipine group ($n = 47$) was 195 (159) μmol/l with a range of 57–923 μmol/l. The difference in means was 1.9 μmol/l ($P = 0.95$, Student's t-test; 95 % confidence interval = –57.8–61.6).

Glomerular filtration rate (GFR) was measured at 6 weeks in 10 recipients in the Eurocollins group and in 13 recipients in the nicardipine group treated in Leicester. The mean (SD) GFR in the Eurocollins group was 45.1 (18.4) ml/min with a range of 13.3–72 ml/min and in the nicardipine group was 42.3 (15.3) ml/min with a range of 22.7–65 ml/min. The difference in means was 2.8 ml/min ($P = 0.6$, 95 % confidence interval = –10.9–16.5).

Rejection episodes

A total of 18 recipients (28 %) in the Eurocollins group experienced a rejection episode in the first 6 weeks post-transplant compared to 17 (27 %) in the nicardipine group ($P = 1.0$, Fisher's exact test).

Adverse events

Four recipients in the Eurocollins group did not complete the study because of adverse events. There was one fatal myocardial infarction, two irreversible acute allograft rejections and one acute graft thrombosis. Similarly, in the nicardipine group, six patients did not complete the study period. There was one episode of fatal pancreatitis, two irreversible allograft rejections, two acute graft thromboses and one irreversible ureteric necrosis.

Discussion

The cellular injury that causes DGF following renal transplantation occurs as a result of two separate influences, the ischaemic anoxia itself and a reperfusion injury component. During cellular anoxia, plasma membrane slow calcium channels open, allowing calcium to run down its concentration gradient into the cytosol. Intra-mitochondrial accumulation of calcium results [6] and this is the pivotal step in ischaemic cell damage. Mitochondrial respiration is inhibited, leading to depletion of cellular ATP and an accumulation of hypoxanthine. At reperfusion the re-introduction of molecular oxygen allows hypoxanthine to be metabolised to xanthine with a rapid generation of oxygen-derived free radicals. These highly unstable and reactive oxidant species are the mediators of reperfusion injury, causing activation of circulating neutrophils [24] with consequent microvascular injury leading to acute tubular necrosis in the kidney and, ultimately, DGF. Calcium channel blockade would be expected to modify this sequence of events and reduce the degree of reperfusion damage. In addition, calcium channel blockers reduce renal vasoconstriction by inhibiting smooth muscle contraction and antagonising angiotensin II [13], and these actions are also of potential benefit.

Various calcium channel blockers, when added to renal perfusion fluids, have been shown to reduce the incidence of DGF in human renal transplantation. Neumayer et al. [15] added diltiazem with or without the prostacyclin analogue iloprost to perfusion fluid in a prospective randomised trial and demonstrated a fall in DGF from 58 % in the control group to 19 % in the diltiazem group. Less than 20 patients were studied in each subgroup and combined treatment with iloprost and diltiazem did not show any additive effect. In another small study, verapamil was both infused into the renal artery at the time of organ retrieval and added to the renal perfusion fluid. The result was a fall in DGF from 33 % to 9 % [18]. Wagner et al. [20, 21] took a different approach, adding diltiazem to Eurocollins solution at donor nephrectomy and, in addition, administering it to the transplant recipient for 2 days post-operatively. Again a significant reduction in the incidence of post-transplant acute tubular necrosis was noted. In all these studies patients were immunosuppressed with cyclosporin. Both diltiazem and verapamil interfere with blood levels of cyclosporin, causing unpredictable increases [5], and this might prove to be a problem in the early post-transplant period.

The present study is the first to use the new channel blocker nicardipinie in human renal transplantation. The addition of this drug to the renal perfusion fluid was not demonstrated to have a beneficial effect on the incidence of DGF, renal function at 6 weeks post-transplant or the incidence of acute rejection during this period. The explanation for this may lie in the fact that nicardipine was only added to the perfusion fluid. Retrospective and prospective human studies have shown that treatment of the recipient alone with oral nifedipine in the immediate pre- and post-transplant priod reduced the incidence of DGF [7–9]. In the present study the use of pre-operative calcium channel blockers was not shown to exert a significant influence on the incidence of DGF. The use of post operative calcium channel blockers did reduce the incidence of DGF from 38 % to 22 %, although this difference did not quite reach statistical significance at the 5 % level ($P = 0.08$). Analysis of sub-groups shows that the reduction in DGF associated with post-operative calcium channel blocker therapy was greater in patients receiving kidneys perfused with Eurocollins solution than those receiving kidneys perfused with nicardipine. Taken together these data suggest that the use of post-operative calcium antagonist therapy is more important than administration of these agents to the donor kidney at the time of perfusion or to the recipient pre-operatively. The reason for the importance of this late protection may be related the finding that intra-mitochondrial calcium concentration reaches its maximum 24 h after reperfusion [25].

In conclusion, this study has demonstrated that the use of nicardipine as an additive to the perfusion fluid alone cannot be recommended as a means of reducing the incidence of delayed graft function in kidney transplants.

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