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Endothelial dysfunction in renal transplant recipients maintained on cyclosporine

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Endothelial dysfunction in renal transplant recipients maintained on cyclosporine.

Background. Hypertension is almost universal following renal transplantation and may contribute to the already poor cardiovascular prognosis of this group. Cyclosporine-induced hypertension is a particular problem and has variously been attributed to increased sympathetic nerve activity, salt and water retention, and increased circulating endothelin levels. However, the effects of cyclosporine on the L-arginine/nitric oxide (NO) system in vivo in humans are unknown. In this present study, we examined basal and stimulated NO production from the vascular endothelium in cyclosporine-treated renal transplant recipients using the technique of forearm venous plethysmography.

Methods. In study 1, stimulated NO production was assessed in 9 cyclosporine-treated renal transplant recipients (CsA), 7 azathioprine-treated renal transplant recipients (AZA), and 12 controls, using carbachol (an endothelium-dependent vasodilator) and sodium nitroprusside (an endothelium-independent vasodilator). In study 2, basal NO production was assessed in 9 cyclosporine-treated patients and 11 controls using L-NMMA (inhibits NO synthase), with norepinephrine as a control vasoconstrictor. Drugs were infused into the nondominant forearm through a sterile 27-gauge needle, and changes in forearm blood flow (FBF) were measured using venous occlusion plethysmography.

Results. In study 1, sodium nitroprusside caused a similar dose-dependent increase in FBF in all groups. However, the median (range) percentage increase FBF to carbachol (3 μg/min) was markedly reduced in the CsA patients (188.8; 72.5 to 385.1) compared with AZA patients (378.1; 124.0 to 548.9; $P = 0.042$) and to controls (303.8; 124.8 to 813.3; $P = 0.028$). In study 2, the maximum percentage reduction in FBF to L-NMMA (4 μmol/min) was less pronounced in CsA patients (−19.5; −4.7 to −63.1) compared with controls (− 39.5; −15.7 to −52.8; $P = 0.056$), and while controls vasoconstricted to the maximum dose of norepinephrine (240 pmol/min) as expected (−26.9; −1.4 to −38.6), CsA patients as a group tended to vasodilate (7.9; −36.8 to 92.6; $P = 0.02$).

Conclusion. These data demonstrate impaired stimulated and basal NO production in CsA patients, indicating endothelial dysfunction. This may predispose patients to atherosclerosis and may be involved in the etiology of post-transplant hypertension.

Key words: renal transplantation, hypertension, cardiovascular disease, forearm plethysmography, vascular endothelium.

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The introduction of cyclosporine to standard immunosuppressive protocols in 1984 has greatly improved graft survival in renal transplantation [1], but patients continue to die prematurely of cardiovascular disease. Figures from the European Renal Association Registry show an approximate tenfold increase in mortality from cardiovascular disease in renal transplant recipients compared with the general population [2]. Hypertension occurs in approximately 80% of patients following renal transplantation [3] and is likely to play a major role in the development of cardiovascular disease; however, the pathophysiology of cyclosporine-induced hypertension is unclear.

In keeping with most forms of hypertension, cyclosporine-induced hypertension involves an increased systemic vascular resistance [4]. Several clinical and in vitro studies have suggested a range of possible mechanisms, including sodium retention [5], raised circulating endothelin levels [6], and altered prostaglandin production [7]. Some studies have demonstrated activation of the sympathetic nervous system [8], while others have not [9, 10]. In animal studies, cyclosporine increases plasma renin activity, while in humans, there is either little change or a reduction in plasma renin activity [11], although renin may be inappropriately high for the degree of blood pressure.

Early organ bath work showed that cyclosporine vasoconstricted smooth muscle preparations, an effect partially blocked by α-adrenoceptor antagonists [12]. More recently, attention has focused on the L-arginine/nitric oxide (NO) system, with work showing attenuated acetylcholine (NO)-mediated vasodilation in cyclosporine-treated dogs [13] and rats [14], and a reduction in inducible NO synthase gene expression in rats [15]. A recent

study in humans failed to show any improvement in endothelium (NO)-dependent vasodilation after conversion from cyclosporine to azathioprine (AZA) [16].

The aim of this study was to examine both basal and stimulated endothelial NO production in cyclosporine-treated renal transplant recipients using the in vivo technique of forearm strain-gauge plethysmography.

METHODS

Subjects

Renal transplant recipients, at least one year post-transplantation and with stable allograft function, were recruited from the transplant follow-up clinic at the Western Infirmary (Glasgow, UK). Controls without renal disease were obtained from hospital staff and by advertisement. All subjects gave written informed consent, and the protocol was approved by the local ethics committee.

In study 1, stimulated NO production was assessed in 9 CsA-treated patients, 7 patients on an AZA-based regime, and 12 controls without renal disease. In study 2, basal NO production was assessed in 9 CsA-treated patients and 11 controls. Patients in the cyclosporine-treated group were maintained on prednisolone (5 to 10 mg/day), AZA (1 to 2 mg/kg/day), and cyclosporine (3 to 5 mg/kg/day). Patients in the AZA group were maintained on prednisolone (5 to 10 mg/day) and AZA (1 to 2 mg/kg/day). These patients were initially also treated with cyclosporine, but this was withdrawn at 6- to 12-months post-transplantation for a variety of reasons, including hirsutism, gingival hyperplasia, gout, and hypertension.

The patient and control groups were of a similar age and sex distribution and had similar smoking habits and serum cholesterol concentrations. Background characteristics of subjects enrolled into study 1 (stimulated NO production) are depicted in Table 1, and of those enrolled into study 2 (basal NO production) are in Table 2. Three of the controls and six of the cyclosporine-treated patients participated in both studies.

Patients were excluded if they were currently anticoagulated, had a history of diabetes mellitus, vasculitis, or myocardial infarction, or were taking anti-anginal medication or statins. Patients were also excluded if they had severe hypertension such that antihypertensive drugs could not be withheld prior to the study.

All subjects were studied in the morning after an overnight fast and were asked to omit aspirin for seven days and antihypertensive medication for at least 48 hours before the study. Immunosuppressive drugs were taken normally on the day of study. Subjects were asked to avoid caffeine and alcohol and to refrain from smoking for at least 12 hours prior to the study.

As stimulated endothelial NO production was normal in AZA-treated patients in study 1, only cyclosporine-treated patients and controls were examined further in study 2.

Plethysmography

All studies were performed in a vascular research laboratory maintained at 24 to 26°C. The room was sealed and quiet and had dimmed lighting. Subjects lay supine with arms supported above heart level on foam blocks. Pediatric cuffs (Hokanson TMC-7; PMS Instruments, Maidenhead, UK) were placed around the wrists and inflated to at least 40 mm Hg above systolic blood pressure for three minutes during each recording, to exclude the hand circulation from the system. Collecting cuffs (Hokanson SC10) were placed around the upper arms and inflated (40 mm Hg) and deflated in a 15-second cycle. Rapid cuff inflation was achieved using a Hokanson AG101 air source, linked to two Hokanson E20 rapid cuff inflators. Blood pressure was measured at the end of each blood flow recording, using a semiautomatic oscillometric sphygmomanometer (Critikon Dinamap Plus, Tampa, FL, USA) placed around the dominant arm, over the collecting cuff.

The maximal circumference of the forearm was measured and a mercury-in-silastic strain gauge (Hokansen forearm set) chosen 2 cm shorter than the circumference. Strain gauges were calibrated electrically while in position using a built-in calibration method.

Under local anesthesia (1 mL 1% lignocaine), a sterile 27-gauge dental needle (Terumo, Japan) was inserted into the brachial artery of the nondominant forearm. The needle was connected to an infusion pump using a modified 16-gauge epidural giving set (Portex, Hythe, UK), and drugs or 0.9% saline was infused throughout the study at 1 mL/min. Baseline measurements of forearm blood flow (FBF) were obtained at 10-minute intervals for 30 minutes to allow acclimatization to inflation and deflation of the cuffs. The final baseline reading was used as the baseline blood flow.

Infusion protocol

In study 1, incremental infusions of carbachol (0.1, 0.3, 1.0, 3.0 μg/min) and sodium nitroprusside (0.3, 1.0, 3.0, 10.0 μg/min) were then commenced, with the order of drugs randomized between subjects. In study 2, norepinephrine was infused first (60, 120, 240 pmol/min) followed by N^G-monomethyl-L-arginine (L-NMMA; 1, 2, 4 μmol/min) in view of its long half-life. There was a 30-minute washout period with 0.9% saline between drug infusions. The protocol is illustrated in Figure 1. All drug solutions were prepared in 0.9% saline in the sterile pharmacy productions unit in the hospital. Each drug was infused for seven minutes with FBF measurements taken from minutes 3 through 6, and blood pressure was recorded in the seventh minute. During each drug infusion, several FBF measurements were made; the

Table 1. Background characteristics for patients and controls in study 1

	Control	AZA	CsA	*P* value[a]	*P* value[b]
Male (total)	8 (12)	6 (7)	7 (9)	0.950	—
Smoker *N*	7	4	4	0.950	—
Age *years*	40 (25–51)	45 (33–51)	36 (24–55)	0.681	—
Urea *mmol/L*	4.4 (2.9–6.6)	6.9 (4.6–10.4)	7.6 (5.7–12.5)	<0.001	0.210
Creatinine *μmol/L*	85 (57–108)	133 (80–223)	143 (111–215)	0.001	0.837
Glucose *mmol/L*	4.7 (4.3–5.6)	4.4 (3.8–6.4)	4.7 (4.3–6.5)	0.805	—
Cholesterol *mmol/L*	5.3 (3.5–6.6)	5.4 (3.6–7.4)	5.6 (3.7–9.3)	0.360	—
Triglyceride *mmol/L*	1.1 (0.6–3.1)	1.4 (0.5–2.8)	2.1 (0.6–5.4)	0.242	—
Hemoglobin *g/dL*	14.5 (12.4–15.6)	13.6 (11.0–15.6)	12.9 (10.6–14.7)	0.020	0.351
SBP *mm Hg*	124 (104–158)	130 (106–155)	158 (127–184)	0.007	0.029
DBP *mm Hg*	71 (50–75)	80 (70–91)	89 (70–99)	0.002	0.054
FBF $mL \cdot 100\ mL^{-1}$	2.95 (1.68–4.98)	2.40 (1.75–3.88)	2.80 (1.67–7.84)	0.474	—
Total RRT *months*	—	232 (112–348)	108 (51–231)	—	0.023
Months since Tx	—	129 (83–312)	48 (14–216)	—	0.142
CCBs *N*	—	1	6	—	0.200
β blockers *N*	—	2	3	—	0.900
Aspirin *N*	—	1	1	—	0.900
Trough CsA level *nmol/L*	—	—	82 (41–121)	—	—

Results are expressed as median (range).

Abbreviations are: FBF, baseline forearm blood flow in infused arm; RRT, renal replacement therapy; CCBs, calcium channel blockers; SBP, systolic blood pressure; DBP, diastolic blood pressure; Tx, transplant.

[a]Represents Kruskall-Wallis or chi-square comparison between three groups

[b]Represents Mann-Whitney or chi-square test between CsA and AZA

Table 2. Background characteristics for patients and controls in study 2

	Control	CsA	*P* value
Male (total)	11 (11)	9 (9)	—
Smoker *N*	5	4	0.980
Age *years*	38 (28–42)	36 (24–55)	0.492
Urea *mmol/L*	4.5 (2.8–6.7)	8.2 (6.0–11.7)	<0.001
Creatinine *μmol/L*	87 (73–100)	157 (90–164)	<0.001
Glucose *mmol/L*	5.0 (4.4–5.2)	5.2 (4.1–6.5)	0.182
Cholesterol *mmol/L*	5.0 (3.1–6.8)	5.9 (3.6–6.5)	0.604
Triglyceride *mmol/L*	1.2 (0.8–1.9)	2.0 (0.8–5.4)	0.211
Hemoglobin *g/dL*	13.9 (12.4–15.5)	13.2 (10.5–13.6)	0.019
SBP *mm Hg*	125 (101–144)	146 (108–169)	0.056
DBP *mm Hg*	72 (56–79)	84 (64–95)	0.002
FBF $mL \cdot 100\ mL^{-1}$	2.82 (1.70–5.20)	3.06 (1.40–5.00)	0.849
Total RRT *months*	—	65 (108–244)	—
Months since Tx	—	20 (51–216)	—
CCBs *N*	—	7	—
β blockers *N*	—	3	—
Aspirin	—	2	—
Trough CsA level *nmol/L*	—	96 (37–161)	—

Results are expressed as median (range). The comparison is by Mann-Whitney or chi-square test.

mean of the final five readings was taken as the FBF achieved for each dose of drug.

Forearm blood flow was expressed as mL blood flow $min^{-1} \cdot 100\ mL^{-1}$ forearm [17]. Bilateral FBF measurements were made, and the results were expressed as a ratio of infused:noninfused arm, as this method is more reproducible than unilateral plethysmography [18] and corrects for background variation in FBF. The response to a particular drug dose was calculated as the percentage change in FBF ratio from baseline.

Laboratory evaluation

Prior to each plethysmography session, a 21-gauge cannula was inserted into the dominant arm, and blood was drawn after a 20-minute period of supine rest. Thirty milliliters of blood were drawn into ethylenediaminetetraacetic acid (EDTA), lithium heparin, or plain tubes, spun, and frozen immediately. In addition to standard hematological and biochemical analyses, assays were performed for renin [19], norepinephrine [20], and for endothelin-1 using a commercially available kit (Cozart Bioscience, UK).

Statistical comparison

All results are expressed as median (range) unless otherwise specified. Comparison between groups is by multivariate analysis of variance with repeated measures. When significant, post hoc analyses were performed by Mann–Whitney U to compare individual groups at each drug dose level. The statistical significance was defined at the 5% level.

All statistics were performed using SPSS package for Windows, version 7.0.

RESULTS

The neurohumoral data from studies 1 and 2 are detailed in Tables 3 and 4, respectively. In study 1, there was a trend toward higher endothelin levels in patients compared with controls. This was significant for AZA-treated patients (0.48 fmol/mL; range 0.10 to 1.15) compared with controls (0.19; 0.01 to 0.49; $P = 0.022$). Renin levels were similar for all groups studied; however, there

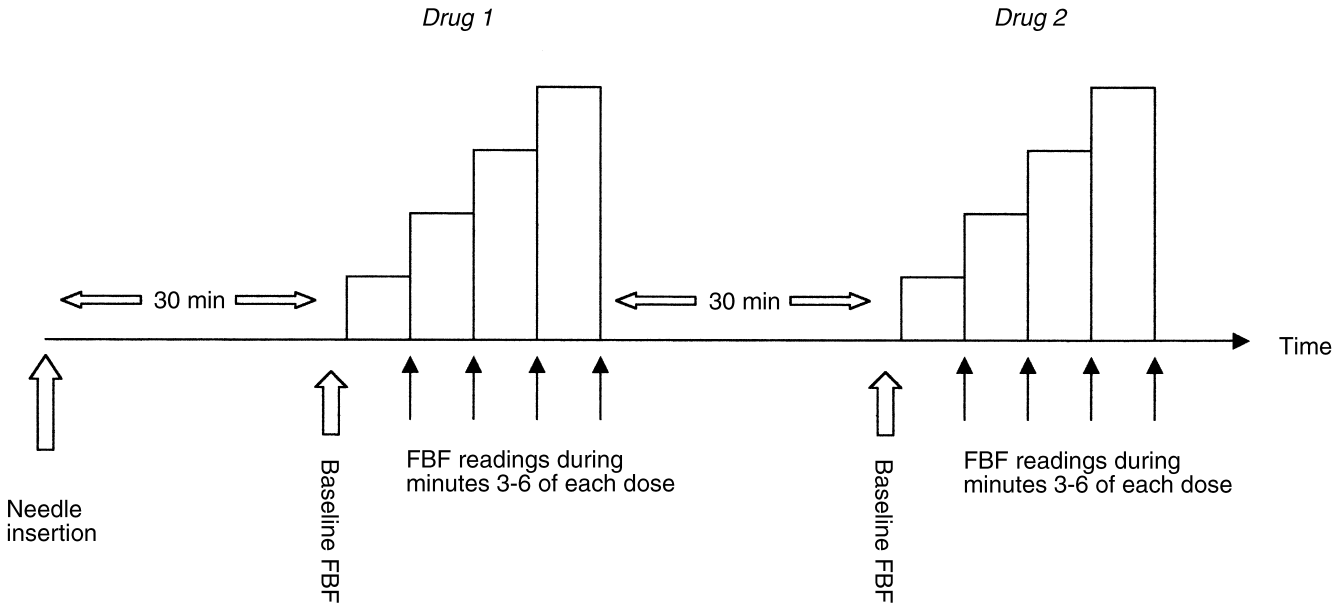

Fig. 1. Infusion protocol for plethysmography. Each dose of drug was infused for seven minutes. Saline or drugs (dissolved in saline) were infused at 1 mL/min for the duration of the study.

Table 3. Neurohumoral data for study 1

	Control	AZA	CsA	*P* value[a]	*P* value[b]	*P* value[c]
Endothelin-1 *fmol/mL*	0.19 (0.01–0.49)	0.48 (0.1–1.15)	0.36 (0.18–2.1)	0.019	0.068	0.397
Renin *uU/mL*	20 (2–44)	27 (16–74)	20 (6–57)	0.104	0.840	0.281
Norepinephrine *nmol/L*	1.15 (0.01–4.20)	0.30 (0.01–5.30)	0.05 (0.01–4.30)	0.596	0.238	0.463

Results are expressed as median (range). Statistical comparison is by Mann-Whitney test.
[a]Between control and AZA
[b]Between control and CsA
[c]Between AZA and CsA

Table 4. Neurohumoral data from study 2

	Control	CsA	*P* value
Endothelin-1 *fmol/mL*	0.12 (0.01–0.30)	0.13 (0.01–0.30)	0.439
Renin *uU/mL*	12.0 (6.0–28.0)	14.0 (7.0–56.0)	0.385
Noradrenaline *nmol/L*	0.70 (0.01–1.90)	0.30 (0.01–1.00)	0.558

Results are expressed as median (range), and comparison is by Mann-Whitney test.

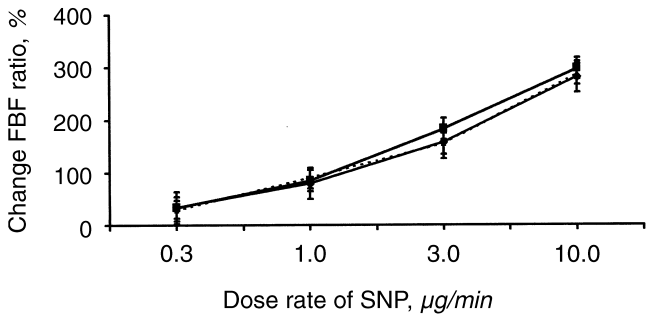

Fig. 2. Dose–response curves for infusion of sodium nitroprusside (study 1), comparing responses in controls (◆), CsA-treated (▲), and azathioprine-treated (■) renal transplant recipients. The results are expressed as mean ± SEM.

was a trend toward lower norepinephrine levels in CsA patients compared with AZA patients and controls.

Results for study 1 are illustrated in dose–response curve form, with results for infusion of sodium nitroprusside (SNP) in Figure 2 and for carbachol in Figure 3. All three groups vasodilated similarly to SNP ($P = 0.985$ by multivariate analysis of variance; MANOVA) demonstrating that endothelium-independent vasodilation is comparable between groups. However, vasodilation to carbachol (Fig. 3) was attenuated in the cyclosporine-treated patients ($P = 0.008$, MANOVA) with a significant reduction in response to 3 μg/min carbachol in the cyclosporine group (188.8; 72.5 to 385.0; % change FBF ratio) compared with controls (303.8; 124.8 to 813.3; $P = 0.028$) and to the AZA group (378.1; 124.0 to 548.9; $P = 0.042$).

In study 2, the response to infusion of L-NMMA is

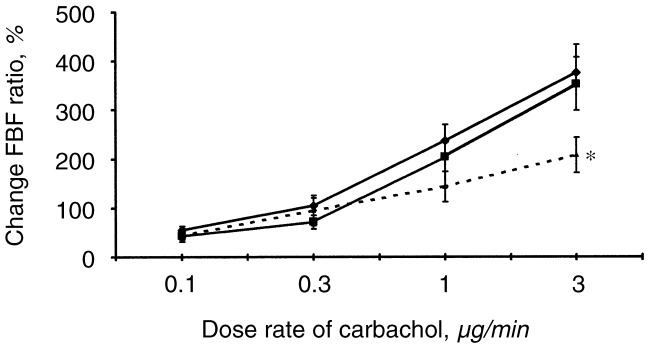

Fig. 3. Dose–response curves for infusion of carbachol (study 1), comparing responses in controls (◆), CsA-treated (▲), and azathioprine-treated (■) renal transplant recipients. The results expressed as mean ± SEM. *$P < 0.05$

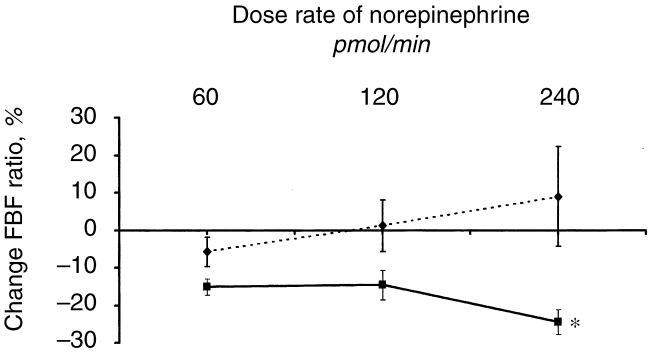

Fig. 5. Dose–response curves for infusion of norepinephrine (study 2), comparing responses in controls (■) and CsA-treated (◆) renal transplant recipients. The results are expressed as mean ± SEM (P = 0.16, MANOVA). *$P < 0.05$

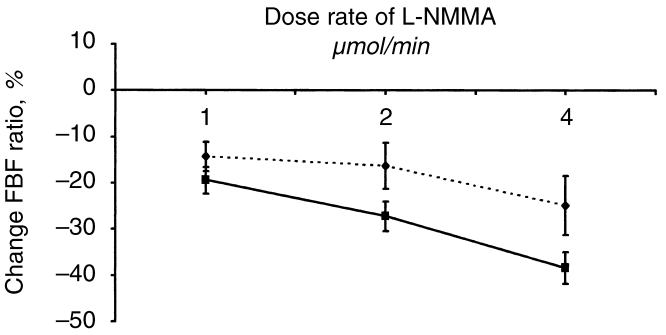

Fig. 4. Dose–response curves for infusion of L-NMMA (study 2), comparing responses in controls (■) and CsA-treated (◆) renal transplant recipients. The results are expressed as mean ± SEM.

illustrated in Figure 4. There was a graphical trend toward reduced vasoconstriction in the cyclosporine group (overall MANOVA, $P = 0.378$), with the difference bordering on statistical significance at 4 μmol/min L-NMMA [−39.4 (−15.7 to −52.8) for controls vs. −19.5 (−4.7 to −63.1) for CsA; $P = 0.053$ by Mann–Whitney test]. The results for the infusion of norepinephrine are illustrated in Figure 5. Cyclosporine patients as a group tended to vasodilate, while controls vasoconstricted as expected ($P = 0.016$, MANOVA). The maximum % change FBF ratio for 240 pmol/min norepinephrine was −27.0 (−0.4 to −38.6) for controls compared with 7.9 (−36.8 to 92.6) for CsA-treated patients ($P = 0.02$, Mann–Whitney test).

Pulse and blood pressure did not change significantly throughout the studies, as the doses of drugs infused were chosen to exert only local effects in the forearm.

DISCUSSION

This study was designed to examine the effects of cyclosporine treatment on basal and stimulated endothelial NO production in humans in vivo. In study 1, cyclosporine-treated renal transplant recipients vasodilated normally to SNP (NO donor), but exhibited attenuated vasodilation to the muscarinic agonist carbachol. This pattern suggests impaired stimulated endothelial NO production. In study 2, the cyclosporine-treated group vasoconstricted less well to the NO synthase inhibitor L-NMMA, suggesting reduced basal endothelial NO production. The results of both studies indicate endothelial dysfunction in cyclosporine-treated renal transplant recipients.

This is unlikely to represent the effects of renal transplantation itself, as AZA-treated patients had preserved stimulated NO production. Neither is it likely to reflect years of uremia prior to transplantation, as the AZA group had a longer total time on renal replacement therapy (Table 1). Endothelial dysfunction has been demonstrated in several conditions, including hypercholesterolemia [21], cigarette smoking [22], diabetes mellitus [23], and cardiac failure [24]. In our study, groups were matched for smoking habit and total cholesterol, although blood pressure was higher in the cyclosporine group. The effects of hypertension on endothelial function are less clear, with one large study showing preserved endothelium-dependent vasodilation [25], and one similarly large study showing abnormal endothelium-dependent vasodilation in essential hypertension [26]. It is therefore possible that the endothelial dysfunction is related to hypertension per se, although the study by Cockcroft et al failed to show endothelial dysfunction in essential hypertension [25]. Another "unmatched" variable was the triglyceride level, although studies in severe hypertriglyceridemia have shown preserved endothelial function [27].

It is thus possible that the reduced endothelial NO production in the cyclosporine-treated patients is a direct effect of cyclosporine therapy, a theory supported by previous in vitro organ-bath work, which demonstrated reduced production of NO from large arteries after short-term exposure to cyclosporine [28]. As NO synthase is

a calcium-calmodulin–dependent enzyme, cyclosporine may directly inhibit the production of NO by inhibition of calcineurin. This may be one potential mechanism contributing to cyclosporine-induced hypertension and may contribute to the high risk of premature atherosclerosis in cyclosporine-treated renal transplant recipients.

A surprising finding of this study was a trend toward reduced vasoconstriction or a slight vasodilation to norepinephrine in cyclosporine-treated patients. In a study using isolated rat aortic strips, cyclosporine added to the organ bath induced a slowly developing contraction, which could be blocked by either the calcium-channel blocker verapamil or by the α-antagonist phenoxybenzamine [12]. It is therefore possible that cyclosporine interferes with α-mediated vasoconstriction in some way, such that infusion of norepinephrine at plethysmography has little effect or vasodilates through the β_2 adrenoceptor. A limitation of study 2 was the absence of a non–CsA-treated group. Thus, it is plausible that the results could reflect years of uremia rather than the effects of cyclosporine. A larger study is warranted to examine the effects of norepinephrine further.

The neurohumoral studies failed to demonstrate significantly raised circulating endothelin levels in the cyclosporine-treated patients, as reported previously [6], perhaps reflecting the small numbers studied. Interestingly, the AZA-treated group tended to have higher endothelin levels, although this failed to alter the response of this group to vasodilators. There was a trend toward lower norepinephrine levels in CsA patients compared with other groups in both studies, in contrast to previous studies in which the norepinephrine level was elevated along with increased sympathetic nerve activity in CsA-treated patients [8]. The reason for the lower norepinephrine levels is unclear and requires further study. Renin levels were lower in study 2, again perhaps reflecting the small numbers studied. There was no difference in diuretic use between studies.

Forearm plethysmography studies are often small, as recruitment of patients for brachial artery cannulation can be difficult. We therefore accept that a limitation of this study is the relatively small study group and that a larger study is warranted to examine the effect of norepinephrine infusion in more detail.

In conclusion, this study demonstrates reduced basal and stimulated NO production from the endothelium of forearm resistance vessels in cyclosporine-treated renal transplant recipients. This suggests endothelial dysfunction, and may explain the increased risk of premature atherosclerosis and cardiovascular death in this group and may provide, at least in part, a potential mechanism to explain cyclosporine-induced hypertension.

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