Cyclosporine A and control of vascular tone in the human forearm: influence of post-transplant hypertension

Christiane Bracht, Xiao-Way Yan, Hans-Peter Brunner LaRocca, Gabor Sütsch and Wolfgang Kiowski

Objective **The use of cyclosporine A after organ transplantation is associated with a high incidence of hypertension, but the underlying mechanisms for this process are not clear. We investigated the effects of blockade of basal release of endothelial nitric oxide and the effects of endothelium-independent and -dependent vasodilators and vasoconstrictors in patients treated with cyclosporine A after heart transplantation.**

Design **We measured blood pressure and forearm blood flow responses to brachial artery infusions of N^G-monomethyl-L-arginine (L-NMMA), sodium nitroprusside, acetylcholine, norepinephrine and vasodilating and vasoconstricting doses of endothelin-1 in eight patients early (< 3 months) and in 11 patients late (> 18 months) after transplantation.**

Results **Diastolic blood pressure was higher late after transplantation, but calculated forearm vascular resistance was lower ($P < 0.01$). Thus, increased forearm vascular resistance does not contribute to the increase in blood pressure. The vasoconstrictor response to L-NMMA was similar in both groups but a reduced endothelium-dependent vasodilator response to acetylcholine was seen late after transplantation. However, impaired smooth muscle responsiveness to nitric oxide may have contributed to this finding, since the response to sodium nitroprusside tended to be reduced. Vasoconstrictor responses to norepinephrine and endothelin-1 were comparable but no vasodilation was seen with low doses of endothelin-1 late compared with early after transplantation ($P < 0.05$).**

Conclusions **The findings in the forearm circulation question the concept of generalized increases in vasoconstrictor responses or a disturbance of tonic, basal release nitric oxide in the pathogenesis of cyclosporine-A-induced hypertension. Although the forearm vasodilator responses to the stimulation of endothelial nitric oxide production and release by acetylcholine, and to low doses of endothelin-1, were impaired, these findings could be explained by the increase in blood pressure rather than cyclosporine A itself. *J Hypertens* 1999, 17:357–363**

Journal of Hypertension 1999, **17**:357–363

Keywords: cyclosporine A, hypertension, cardiac transplantation, acetylcholine, L-NMMA, norepinephrine, endothelin-1

Heart Failure and Transplantation Unit, Division of Cardiology, University Hospital, Zürich, Switzerland.

This work was supported by research Grant BR1423/2-1 (C. B.) from the National Research Foundation of Germany and by Swiss National Foundation Grant (No. 3200-043612. 95/1).

Correspondence and requests for reprints to Professor W. Kiowski, Division of Cardiology, University Hospital, Rämistr. 100, 8091 Zürich, Switzerland. Tel: +41 1 2552358; fax: +41 1 2554401; e-mail: karkiw@usz.unizh.ch

Received 24 June 1998 **Revised** 2 November 1998
Accepted 17 November 1998

Introduction

The discovery and clinical use of cyclosporine A (CsA) has greatly improved the success of organ transplantation [1]. However, its use is associated with a number of adverse effects, among them the development of hypertension [1,2]. Thus, the incidence of hypertension has increased from less than 20% in the pre-CsA era to more than 90%, and has become an important problem in the care of patients after solid organ transplantation [1–3]. The mechanisms behind CsA-induced hypertension are not clear but renal [4], central nervous system [5,6] and vascular smooth muscle effects [7] have been implicated. In addition, studies in animals [8,9] and *in vitro* [10] have suggested that CsA may influence various regulatory functions of the endothelium, among them receptor-operated, nitric oxide (NO)-dependent vasodilation and, thereby, cause or contribute to CsA-induced hypertension. However, in normal volunteers acute administration of CsA enhanced both basal and receptor-stimulated NO activity in the forearm vascular bed [11] and switching hypertensive patients after renal transplantation from CsA to azathioprine did not change receptor-stimulated NO activity in the forearm vasculature [12]. Because of these contradictory results between experimental and human data, we studied the effects of blockade of endothelium-derived basal release of NO, as well as the effects of endothelium-independent and -dependent vasodilators and vasoconstrictors in patients treated with CsA after heart transplantation. These studies were performed either early, i.e. when hypertension has not yet developed, or late after transplantation, i.e.

when blood pressure had increased, to allow a differentiation of the effects of CsA from those of increased blood pressure on these parameters.

Methods

Nineteen male patients were studied following cardiac transplantation. Eight of them (group 1) were investigated early after heart transplantation, i.e. between 2 weeks and 3 months, and 11 patients (group 2) were investigated late after heart transplantation, i.e. between 1.5 and 2.5 years. The protocol was approved by the ethics committee on the use of human subjects in clinical investigations of the University Hospital, Zürich, and written informed consent to participate in the study was obtained from all patients.

Design

Any antihypertensive, but not lipid-lowering, therapy was discontinued for 2 weeks before the study. Investigations were performed with patients recumbent and resting in a quiet, temperature-controlled room and lasted 5–6 h. Patients were allowed a light breakfast and regular immunosuppressive therapy was given in the morning. However, all patients had abstained from ingesting coffee and from smoking cigarettes for at least 8 h before the start of the investigations. Before the study, forearm volume was measured by volume displacement [13].

An 18-gauge catheter (Abbocath-T, Abbott) was inserted under local anesthesia (1% lidocaine) into the left brachial artery of the subjects, for regional drug infusion and recording of arterial pressure. Subjects were allowed to rest for 30 min after instrumentation had been completed.

Then, baseline measurements of forearm blood flow (FBF), intra-arterial blood pressure (Novatrans II transducer; Medex Inc., USA, coupled to a HP M1166A patient monitor; Hewlett-Packard, USA) and heart rate (ECG) were performed. Measurements during drug infusions were always performed during the last minute of each infusion. Drug dosages were chosen that produced only regional but no systemic effects, on the basis of previous studies [13,14].

Following baseline measurements, the endothelium-independent vasodilator sodium nitroprusside (SNP) was infused (0.6 μg/min per 100 ml forearm tissue) for 3 min and, after a washout period of 20–30 min, the endothelium-dependent vasodilator acetylcholine was infused in four increasing dosages (0.8, 10, 40, and 160 μg/min per 100 ml) for 3 min each. After a further washout period of 20–30 min, the effects of inhibition of NO synthesis were investigated by infusing the NO synthase blocker N^G-monomethyl-L-arginine (L-NMMA; 200 μg/min per 100 ml) for 5 min. In order to reverse the effects of the L-NMMA, L-arginine was then infused (850 μg/min per 100 ml for 7 min) followed by a waiting period of 45–60 min. Subsequently norepinephrine was infused in three ascending dosages (7.5, 20, and 40 ng/min per 100 ml) for 5 min each. Finally, after FBF had returned to control values, endothelin-1 was infused in five ascending dosages (0.25, 0.5, 5, 25, and 50 ng/min per 100 ml) for 5 min each. The same sequence of administration was used in all subjects.

Measurements

FBF was measured bilaterally, using a mercury-in-silastic strain gauge plethysmograph with the venous occlusion technique [13]. The strain gauge was placed approximately 5 cm below the elbow on the forearm and coupled to an electronically calibrated plethysmograph (EC4; Hokanson). Venous occlusion was achieved by a blood pressure cuff applied proximal to the elbow and inflated to 40 mmHg with a rapid cuff inflator (EC10; Hokanson). Circulation to the hand was arrested by inflating a cuff around the wrist to 50 mmHg above systolic pressure at least 1 min before the measurements to eliminate the influence of arteriovenous shunts. Experiments were performed on the left (experimental) forearm; blood flow measurements on the right (control) arm served as a control for potential systemic drug effects. The FBF in the experimental arm preceding each intervention was used as a control value for the respective intervention. Plethysmographic recordings were analyzed using a digitizing board and a suitably programmed computer. The mean value of four recordings obtained within 1 min was taken for statistical evaluation. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure, obtained immediately after flow measurements, by FBF and is expressed in arbitrary units (U).

Preparation of solutions

All solutions were freshly prepared before each study. SNP was dissolved in 5% dextrose and protected from light. Acetylcholine, L-arginine and norepinephrine were diluted in 0.9% saline. Endothelin-1 (Clinalfa, Switzerland) and L-NMMA (Sigma, Switzerland) were dissolved in Physiogel (gelatine solution 4%; molecular weight 22 000) to avoid binding to syringes or tubes. Physiogel, in the volumes used in this study, does not affect FBF [15]. Infusions were performed using constant speed infusion pumps (Perfusor Secura FT; B. Braun, Germany) with volume rates between 30 and 90 ml/h.

Statistical analysis

Results are expressed as mean ± SD. Differences between the two groups were analyzed using the Mann–Whitney U-test. Profile analysis of variance for repeated measurements was used to determine the differences

between the two patients groups regarding changes in FBF or FVR during the various drug infusions.

Results

The baseline characteristics of both patient groups are shown in Table 1. Two patients in each group had a history of hypertension. As expected, intra-arterial and ambulatory sitting blood pressures were higher in patients late after transplantation following the withdrawal of antihypertensive therapy for 2 weeks, but the differences between the groups were statistically significant only for diastolic pressure. Consistent with clinical practice aimed at reducing immunosuppressive therapy late after transplantation, steroid doses and CsA plasma levels were significantly lower, while serum levels of creatinine tended to be higher, in patients late after transplantation. Six patients late after transplantation received lipid-lowering drugs but total and high density lipoprotein cholesterol levels were similar in both groups (Table 1).

Table 1 **Patient characteristics**

	Patients early after transplantation ($n = 8$)	Patients late after transplantation ($n = 11$)
Age (years)	51.2 ± 7.3	55.8 ± 7.4
Sitting cuff pressure (mmHg)		
Systolic	146.7 ± 13.4	159.4 ± 20.9
Diastolic	81.9 ± 10.5	$98.4 \pm 13.1^{**}$
Recumbent intra-arterial pressure (mmHg)		
Systolic	140.2 ± 10.2	149.4 ± 18.9
Diastolic	71.3 ± 8.2	$87.1 \pm 11.9^{*}$
Heart rate (beats/min)	87.0 ± 6.7	95.4 ± 8.1
Serum cholesterol (mmol/l)		
Total	5.0 ± 1.1	4.9 ± 0.7
HDL	1.6 ± 0.7	1.2 ± 0.3
Serum creatinine (μmol/l)	103 ± 31	128 ± 19
CsA blood level (μg/l)	224 ± 52	$166 \pm 30^{*}$
Prednisone (mg/day)	24 ± 11	$6 \pm 4^{**}$
ACE inhibitors (*n*)	4	6
Calcium autogonists (*n*)	0	4
Beta-blockers (*n*)	0	1
Lipid-lowering drugs (*n*)	0	6

Values are means ± SD. HDL, high-density lipoprotein; CsA, cyclosporine A; ACE, angiotensin-converting enzyme. $^{*}P < 0.05$; $^{**}P < 0.01$.

Hemodynamic measurements preceding the various pharmacologic interventions (e.g. pre sodium nitroprusside) showed no statistical differences compared with baseline measurements (Table 2), indicating that the waiting periods were sufficient for the drug effects of the preceding intervention to wear off.

Baseline FBF in the experimental arm in group 1 patients was significantly lower than that in group 2 patients (3.4 ± 1.0 versus 6.0 ± 2.3 ml/min per 100 ml; $P < 0.01$), a finding similar to the observation in the control arm (4.1 ± 1.4 versus 6.3 ± 2.2 ml/min per 100 ml; $P < 0.05$). Thus, despite the higher blood pressure, FVR was lower at baseline in patients late after transplantation (29.6 ± 8.2 versus 21.1 ± 6.1 U; $P < 0.05$).

The response to brachial artery infusion of the endothelium-independent nitrovasodilator SNP was not statistically different in groups 1 and 2, with FBF increasing to 10.4 ± 2.3 and 11.5 ± 2.6 ml/min per 100 ml, respectively. However, both absolute and percentage increases in FBF in response to the endothelium-dependent vasodilator acetylcholine were significantly attenuated in patients late after transplantation (Fig. 1). In these patients, FBF increased to a maximum of 17.9 ± 3.5 ml/min per 100 ml compared with 24.0 ± 4.0 ml/min per 100 ml early after transplantation ($P < 0.05$). Thus, when taking into account the differing baseline flow values, FBF increased $582 \pm 220\%$ early and $252 \pm 109\%$ late after transplantation ($P < 0.001$). Similarly, the reduction in FVR was attenuated in patients late after transplantation and decreased with the highest acetylcholine dose from 29.3 ± 8.3 to 4.3 ± 1.1 U (a decrease of 25.0 ± 3.0 U) in group 1 and from 22.2 ± 9.1 to 6.3 ± 1.3 U (a decrease of 16.0 ± 3.6 U) in group 2 ($P < 0.05$ by repeated measures analysis of variance for changes with the four acetylcholine doses).

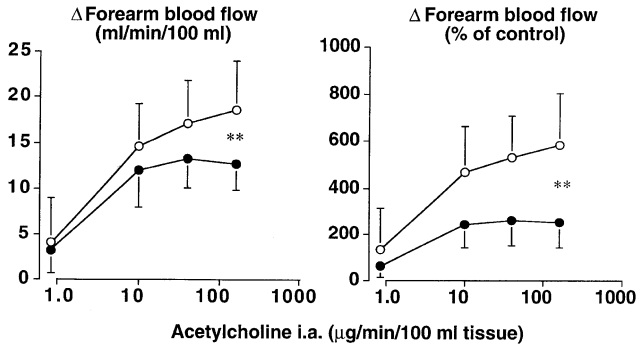
Because lipid-lowering drugs improve endothelium-

Table 2 **Hemodynamic data obtained before the various pharmacological interventions in 19 heart transplant patients**

	FBF (ml/min per 100 ml tissue)	Mean blood pressure (mmHg)	FVR (units)	Heart rate (beats/min)
Baseline/pre SNP	5.0 ± 2.3	103.3 ± 21.1	24.6 ± 8.1	91.2 ± 20.8
Pre-acetylcholine	4.9 ± 2.3	104.8 ± 14.6	24.8 ± 9.3	92.5 ± 19.9
Pre-L-NMMA	5.2 ± 1.9	106.2 ± 15.1	23.0 ± 8.4	92.0 ± 20.1
Pre-norepinephrine	5.3 ± 1.9	107.6 ± 15.3	23.3 ± 8.5	93.0 ± 21.8
Pre-endothelin-1	5.5 ± 1.8	108.4 ± 14.1	22.8 ± 8.5	92.7 ± 20.0

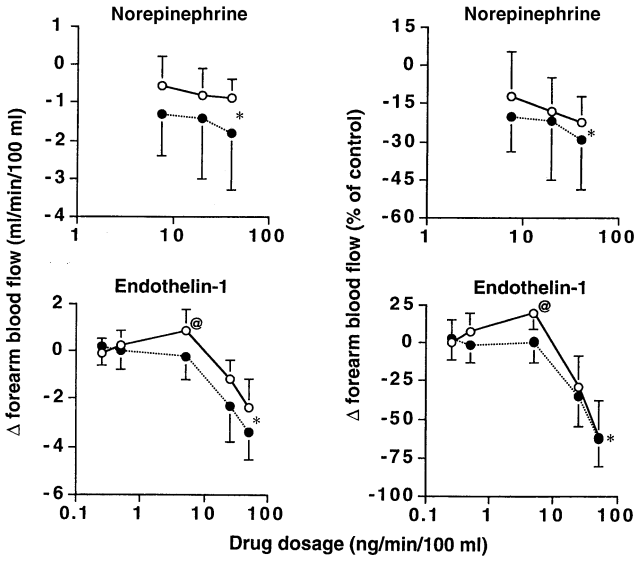
Values are means ± SD. FBF, forearm blood flow; FVR, forearm vascular resistance; SNP, sodium nitroprusside; L-NMMA, N^{G}-monamethyl-L-arginine.

Fig. 1



Forearm blood flow responses to brachial artery infusions of acetylcholine in eight patients early and 11 patients late after heart transplantation. Absolute changes are depicted in the left and percentage changes from baseline in the right panel. Open circles represent patients early and closed circles patients late after transplantation. $^{**}P < 0.01$, two-way analysis of variance for repeated measurements. Values are means ± SD. i.a., intra-arterially.

Fig. 2



Forearm blood flow responses to brachial artery infusions of norepinephrine (upper panels) and endothelin-1 (lower panels) in patients early (open circles) and late (closed circles) after transplantation. Absolute changes are shown in the left and percentage changes from baseline in the right panels. $^{*}P < 0.05$ for within-group comparison of norepinephrine and endothelin-1 effects versus control values. @$P < 0.05$ for comparison of low-dose endothelin-1 effect in patients studied early and late after transplantation. Values are mean ± SD.

dependent vasodilation in hypercholesterolemic patients [16], the responses to acetylcholine were also analyzed separately for patients late after transplantation who were receiving lipid-lowering drugs ($n = 6$) or not ($n = 5$). No difference was found between these subgroups, in whom FBF increased similarly in response to acetylcholine, and by a maximum of 11.7 ± 3.2 ($259 \pm 141\%$) and 14.9 ± 2.1 ($244 \pm 83\%$) ml/min per 100 ml (NS) in patients treated or not treated with lipid-lowering drugs, respectively.

The response to blockade of basal NO production by L-NMMA was similar in both groups: FBF decreased by 1.7 ± 1.1 ml/min per 100 ml ($31 \pm 12\%$) in group 1 and by 2.4 ± 1.9 ml/min per 100 ml ($30 \pm 16\%$) in group 2 patients.

The absolute and percentage changes in FBF in response to infusions of norepinephrine and endothelin-1 are shown in Figure 2. Both groups showed similar decreases in FBF in response to norepinephrine (Fig. 2, upper left panel); relative changes from the respective baselines were likewise similar (Fig. 2, upper right panel).

The vasoconstrictor response to the highest dose of endothelin-1 did not differ between both groups (Fig. 2, lower panels). However, low-dose endothelin-1 caused a small but significant increase in FBF only in patients early after transplantation (Fig. 2, lower panels). Thus, patients showed a $15 \pm 18\%$ increase in FBF early after transplantation, while only a non-significant degree of vasodilation ($3 \pm 13.5\%$) was observed for low-dose endothelin-1 in patients late after transplantation (Fig. 2, lower right panel).

FBF in the control arm and blood pressure did not change significantly during the study, indicating that no systemic drug effects occurred at the dosages used.

Discussion

The use of CsA for the prevention of graft rejection after organ transplantation leads, in the majority of patients, to an increase in blood pressure [1–3] through an increase in systemic vascular resistance [17] but the mechanisms underlying this adverse effect are not clear. Several mechanisms have been proposed, among them changes in the renal circulation [4], increased sympathetic nerve activity [5,6], an increase in vascular smooth muscle responsiveness to sympathetic nerve stimulation or exogenously administered norepinephrine, possibly through a direct vascular smooth muscle effect [7], increased endothelin-1 mediated vasoconstriction [18], and a decrease in the vasodilatory capacity of the endothelium [9]. However, most of these data were obtained after acute administration of CsA either in-vitro or in animal experiments and little is known about the effects of CsA on these parameters in humans.

Our findings demonstrate that no increase in vascular responsiveness to norepinephrine or endothelin-1 occurs late after transplantation when blood pressure is

elevated in most CsA-treated patients. Moreover, there was no evidence for a defect in basal NO-mediated control of tone in the FVR vessels of patients with CsA-induced increases in blood pressure, compared with normotensive patients. Furthermore, we could confirm that vasodilation occurs in the forearm vascular bed, since basal FBF was increased and FVR decreased in patients with CsA-induced increases in blood pressure [19], in contrast to the vasoconstriction reported in the human calf [5]. Acetylcholine-induced vasodilation was significantly reduced in patients late after transplantation, a finding which is similar to that found in some, but not all, patients with primary [13,20] and secondary [21] hypertension. Finally, the vasodilator response to low-dose endothelin-1 was absent in transplanted patients with CsA-induced increases in blood pressure. A number of points relating to these findings need comment.

Difference in baseline hemodynamics

Basal FBF was increased in patients with CsA-induced increases in blood pressure. Similarly, FBF increased and vascular resistance decreased in another study following the ingestion of CsA in heart transplant recipients investigated on average 49 weeks after transplantation [19]. Although we did not measure systemic vascular resistance and, therefore, cannot be absolutely sure that increased blood pressure was due to an increase in systemic vascular resistance, others have found CsA-induced hypertension to be characterized by increased systemic vascular resistance [17]. Our observation of increased baseline FBF and reduced FVR therefore imply that vascular resistance must be increased in other vascular beds. This pattern of increased skeletal muscle perfusion and presumably decreased splanchnic and renal perfusion [19] resembles to some extent that seen in normal subjects during mental stress, preparation for physical work, and during muscular exercise. However, cardiac output, which is increased under these circumstances [22] is normal in CsA-induced hypertension [17]. Such hemodynamic changes have been ascribed to a common central nervous system reaction seen in any defense situation in preparation for self-preservation, i.e. the 'fight and flight' reaction [22]. A central nervous system effect of CsA appears to be an attractive, although speculative, explanation. We have no explanation for the finding that CsA decreased muscle flow in the human calf [5], although different muscular beds may respond differently to various stimuli [19,23].

Interestingly, brachial artery infusion of CsA in normal volunteers did not affect FBF but enhanced the decrease of FBF when NO synthesis was blocked by L-NMMA, suggesting a compensatory increase in NO activity as a result of CsA [11]. The similar response of FBF to NO synthase blockade by L-NMMA argues against this explanation as a mechanism for the increased basal FBF in our patients late after transplantation. Obviously, the effects of direct brachial artery infusion of CsA in normal volunteers may differ from chronic oral administration of CsA in transplant patients. Our data are also somewhat at variance with results obtained after switching renal transplant patients from CsA to azathioprine [12]. While blood pressure decreased in these patients, FBF fell only insignificantly after switching. However, levels of CsA appeared to be lower in those patients than in ours, although a direct comparison is not possible because of the use of a polyclonal assay in that study, and a monoclonal assay in ours. Moreover, antihypertensive drugs were discontinued for at least 2 weeks in our patients, compared with 3 days. Thus, differences in patient characteristics and methods might explain why FBF showed a tendency to decrease only after switching from CsA to azathioprine [12].

Vascular responsiveness to vasoconstrictor stimuli

Studies in animals and in-vitro have suggested that vasoconstrictor responses are augmented by CsA [9,10]. At the cellular level, this effect seems to be mediated by a CsA-induced increase in transmembraneous uptake of smooth muscle Ca^{2+}, which increases the availability of trigger calcium in smooth muscle cells [7]. Although such an effect might be expected to be present throughout the circulation, we were unable to demonstrate differences in the responses to two different vasoconstrictor agents, norepinephrine and endothelin-1, in patients with and without CsA-induced increases in blood pressure in the human forearm model. Thus, these findings seem to argue against the importance of such an effect in the development of hypertension. However, we cannot exclude the possibility that forearm resistance vessels respond differently to norepinephrine than do, for example, renal vessels, as has been described after acute administration of CsA in rats [24].

Endothelial vasodilator control of vascular tone

The blockade of basal endothelium-derived NO by L-NMMA did not reveal a difference in FBF changes in response to L-NMMA between patients with and without post-transplant hypertension either in absolute or in relative terms. In contrast, the vasodilator response to acetylcholine, known to be predominantly mediated through the endothelial release of NO [25], was significantly attenuated in patients late after transplantation. The similar response to the direct smooth muscle relaxing agent SNP suggests that the impaired response to acetylcholine is related to a defect in the endothelial production, release or availability of NO, rather than to a disturbed smooth muscle response to guanylyl cyclase stimulation in patients late after heart transplantation. However, the results obtained with SNP have to be

interpreted with some caution because we did not assess SNP dosages that would have resulted in flow rates similar to those seen for the higher acetylcholine doses. Nevertheless, the reduced response to acetylcholine is similar to several in-vitro and animal experiments, which have demonstrated in a variety of vascular beds that endothelium-dependent vasorelaxation may be impaired in the presence of CsA [8,9]. This finding also resembles observations in some but not all patients with primary hypertension, who exhibited a similarly blunted response to acetylcholine [13,20,21]. Thus it is possible that this finding may be related to increased blood pressure rather than to CsA itself. There is reason to favor the latter contention. Thus, levels of CsA were higher in patients early after transplantation, but the acetylcholine response was greater than in patients late after transplantation. Although it might be argued that prolonged exposure to CsA is required for the development of this endothelial defect, the reduction in acetylcholine response *in vitro* and in animals has usually been seen after acute administration of CsA. Therefore, it appears likely that this finding in humans is related to the increase in blood pressure rather than to direct effects of CsA on the endothelium. Thus, CsA-induced hypertension appears to be similar to other forms of secondary hypertension which also show decreased acetylcholine-mediated vasodilation of forearm resistance vessels [26].

Hypercholesterolemia impairs endothelium-mediated NO release following muscarinic receptor stimulation [27] and the majority of our patients required lipid-lowering therapy during long-term follow-up. However, cholesterol levels did not differ between patients early and late after transplantation. In addition, patients late after transplantation receiving lipid-lowering drugs had a somewhat, though not significantly, smaller response to acetylcholine than those not receiving lipid-lowering drugs. Because lipid-lowering therapy restores impaired NO-mediated vasodilation in hyperlipidemic patients [16], it might be argued that the acetylcholine response in our patients would have been even more impaired without lipid-lowering therapy. For this reason, it appears unlikely that this difference in therapy explains our observations.

Patients with CsA-induced increases in blood pressure did not respond to low-dose endothelin-1 infusion with vasodilation. This vasodilator effect, mediated by activation of endothelin type B receptors on endothelial cells, appears to be the result of endothelial release of prostacyclin or NO, or both [28,29]. A suppression of the generation of endothelial prostacyclin by CsA has been described in isolated human umbilical veins in culture [30]; this finding may therefore indicate an endothelial defect related to CsA. However, a non-specific effect related to the increase in blood pressure cannot be excluded [5,19,24,31–33].

Limitations of the study

We used the forearm infusion model with a fixed sequence of drug administration. Although time was allowed for FBF to return to control values after an intervention, it cannot entirely be ruled out that drug interactions influenced our findings, except for baseline measurements. Differing baseline FBF values also influence the effective intra-arterial concentration of a drug. Thus, the reduced response to acetylcholine late after transplantation might have been, at least in part, the result of higher baseline FBF and, therefore, reduced concentrations of acetylcholine. However, the differences with respect to acetylcholine-induced vasodilation were most marked for the higher concentrations. when the difference between absolute FBF values in both groups were only approximately 25% compared with almost 50% at baseline. Similarly, the missing vasodilator response to low-dose endothelin-1 might have been influenced by such an effect. In addition, the response to SNP either tended to be smaller (when analyzed as absolute FBF changes) or was significantly reduced (when analyzed as percentage changes from the higher baseline values) in patients late after transplantation. Since we did not assess whether this difference persisted at flow rates similar to those seen for the higher acetylcholine doses, we cannot exclude the possibility that a reduced smooth muscle responsiveness to NO stimulation contributed to the reduced response to acetylcholine. However, in-vitro and animal experiments suggest that smooth muscle responsiveness to NO is not impaired by CsA [34,35]. Finally, the interpretation of findings derived using this technique must be made with caution, because differences between this vascular bed, which supplies mostly muscle and skin, and others can not be excluded.

Conclusions

Our findings argue against there being a generalized increased vasoconstrictor response or a disturbance of tonic, basal release of NO in the pathogenesis of CsA-induced blood pressure increases in patients late after cardiac transplantation. The vasodilator response to stimulation of the production or release of endothelial NO by acetylcholine and low doses of endothelin-1 were impaired. However, these changes are more likely to have been caused by the increase in blood pressure than by the effects of CsA itself, and appear similar to those described, at least for the acetylcholine response, in primary and secondary forms of hypertension. However, we cannot exclude the possibility that an impaired smooth muscle responsiveness to NO contributed to the impaired vasodilator response to acetylcholine and that differences exist between the vascular bed studied,

i.e. forearm resistance vessels, and other beds regarding the mechanisms investigated.

References

1 Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB, *et al.* Cyclosporine: a new immunosuppressive agent for organ transplantation. *Ann Intern Med* 1984; **101**:667–682.
2 Textor SC. De novo hypertension after liver transplantation [clinical conference]. *Hypertension* 1993; **22**:257–267.
3 Thompson ME, Shapiro AP, Johnsen AM, Itzkoff JM, Hardesty RL, Griffith BP, *et al.* The contrasting effects of cyclosporin-A and azathioprine on arterial blood pressure and renal function following cardiac transplantation. *Int J Cardiol* 1986; **11**:219–229.
4 Luke RG. Mechanism of cyclosporine-induced hypertension. *Am J Hypertens* 1991; **5**:A79–A84.
5 Scherrer U, Vissing SF, Morgan BJ, Rollins JA, Tindall RS, *et al.* Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. *N Engl J Med* 1990; **323**:693–699.
6 Morgan BJ, Lyson T, Scherrer U, Victor RG. Cyclosporine causes sympathetically mediated elevations in arterial pressure in rats. *Hypertension* 1991; **18**:458–466.
7 Meyer-Lehnert H, Schrier RW. Potential mechanism of cyclosporine A-induced vascular smooth muscle contraction. *Hypertension* 1989; **13**: 352–360.
8 Diederich D, Yang Z, Lüscher TF. Chronic cyclosporine therapy impairs endothelium-dependent relaxation in the renal artery of the rat. *J Am Soc Nephrol* 1992; **2**:1291–1297.
9 Roullet JB, Xue H, McCarron DA, Holcomb S, Bennett WM. Vascular mechanisms of cyclosporin-induced hypertension in the rat. *J Clin Invest* 1994; **93**:2244–2250.
10 Bunchman TE, Brookshire CA. Cyclosporine-induced synthesis of endothelin by cultured human endothelial cells. *J Clin Invest* 1991; **88**:310–314.
11 Stroes ES, Lüscher TF, de Groot, FG, Koomans HA, Rabelink TJ. Cyclosporin A increases nitric oxide activity in vivo. *Hypertension* 1997; **29**:570–575.
12 van den Dorpel MA, van den Meiracker AH, Lameris TW, Weimar W, Man in't Veld AJ. Forearm vasorelaxation in hypertensive renal transplant patients: the impact of withdrawal of cyclosporine. *J Hypertens* 1998; **16**:331–337.
13 Linder L, Kiowski W, Buhler FR, Lüscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation *in vivo*. Blunted response in essential hypertension. *Circulation* 1990; **81**:1762–1767.
14 Kiowski W, Linder L, Stoschitzky K, Pfisterer M, Burckhardt D, Burckhardt F, *et al.* Diminished vascular response to inhibition of endothelium-derived nitric oxide and enhanced vasoconstriction to exogenously administered endothelin-1 in clinically healthy smokers. *Circulation* 1994; **90**:27–34.
15 Bolli P, Muller FB, Linder L, Raine AE, Resink TJ, Erne P, *et al.* The vasodilator potency of atrial natriuretic peptide in man. *Circulation* 1987; **75**:221–228.
16 Stroes ES, Koomans HA, de Bruin TW, Rabelink TJ. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. *Lancet* 1995; **346**:467–471.
17 Ventura HO, Milani RV, Lavie CJ, Smart FW, Stapleton DD, Toups TS, *et al.* Cyclosporine-induced hypertension. Efficacy of omega-3 fatty acids in patients after cardiac transplantation. *Circulation* 1993; **88 (suppl II)**:II281–II285.
18 Bartholomeusz B, Hardy KJ, Nelson AS, Phillips PA. Bosentan ameliorates cyclosporin A-induced hypertension in rats and primates. *Hypertension* 1996; **27**:1341–1345.
19 Kaye D, Thompson J, Jennings G, Esler M. Cyclosporine therapy after cardiac transplantation causes hypertension and renal vasoconstriction without sympathetic activation. *Circulation* 1993; **88**:1101–1109.
20 Panza JA, Quyyumi AA, Brush JJ, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; **323**:22–27.
21 Taddei S, Virdis A, Mattei P, Salvetti A. Vasodilation to acetylcholine in primary and secondary forms of human hypertension. *Hypertension* 1993; **21**:929–933.
22 Brod J, Fencl V, Hejl Z, Ulrych M. Circulatory changes underlying blood pressure elevation during acute stress (mental arithmetic) in normotensives and hypertensive subjects. *Clin Sci* 1958; **18**:269–279.
23 Wallin BG. Human sympathetic nerve activity and blood pressure regulation. *Clin Exp Hypertens* 1989; **A11**:91–101.
24 Garr MD, Paller MS. Cyclosporine augments renal but not systemic vascular reactivity. *Am J Physiol* 1990; **258**:F211–F217.
25 Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; **327**:524–526.
26 Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fesolo CB, *et al.* Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation* 1995; **91**:1981–1987.
27 Creager MA, Cooke JP, Mendelsohn ME, Gallagher SJ, Coleman SM, Loscalzo F, *et al.* Impaired vasodilation of forearm resistance vessels in hypercholesterolemic humans. *J Clin Invest* 1990; **86**:228–234.
28 Lüscher TF. Endothelin, endothelin receptors, and endothelin antagonists. *Curr Opin Nephrol Hypertens* 1994; **3**:92–98.
29 La M, Reid JJ. Endothelin-1 and the regulation of vascular tone. *Clin Exp Pharmacol Physiol* 1995; **22**:315–323.
30 Voss BL, Hamilton KK, Samara EN, McKee PA. Cyclosporine suppression of endothelial prostacyclin generation. A possible mechanism for nephrotoxicity. *Transplantation* 1988; **45**:793–796.
31 Bloom IT, Bentley FR, Spain DA, Garrison RN. An experimental study of altered nitric oxide metabolism as a mechanism of cyclosporin-induced renal vasoconstriction. *Br J Surg* 1995; **82**:195–198.
32 Weir MR, Klassen DK, Shen SY, Sullivan D, Buddemeyer EU, Handwerger B. Acute effects of intravenous cyclosporine on blood pressure, renal hemodynamics, and urine prostaglandin production of healthy humans. *Transplantation* 1990; **49**:41–47.
33 Sturrock ND, Lang CC, Clark G, Struthers AD. Acute haemodynamic and renal effects of cyclosporin and indomethacin in man. *Nephrol Dial Transplant* 1994; **9**:1149–1156.
34 Mathieu P, Carrier M, Dupuis J, Ryan J, Pelletier LC. L-arginine prevents cyclosporin A-induced pulmonary vascular dysfunction. *Ann Thorac Surg* 1997; **64**:414–420.
35 Sudhir K, MacGregor JS, DeMarco T, De GC, Taylor RN, Chou TM, *et al.* Cyclosporine impairs release of endothelium-derived relaxing factors in epicardial and resistance coronary arteries. *Circulation* 1994; **90**:3018–3023.