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Six-month cardiovascular changes in cyclosporine-treated recipients of corneal grafts: serial baroreflex responses

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Abstract Loss of autonomic cardiovascular homoeostasis is often suspected in hypertension that has developed after organ transplantation. We serially assessed autonomic cardiovascular control in eight patients in generally good condition who needed cyclosporine (CyA) treatment because of high risk of rejection in corneal transplantation. To this aim, we investigated the patients, before and while they were receiving CyA, for 1 week and at 1, 3 and 6 months. For each period, spontaneous baroreflex, blood pressure, heart rate and variability were assessed, as well as neuro-hormonal indicators, while the patients were in both supine and 60° upright positions. After 1 week of treatment, patients showed increases in systolic, diastolic and pulse blood pressures ($P < 0.05$) that were concomitant with a transient decrease in plasma noradrenaline ($P < 0.05$), which, thereafter, resumed the range of baseline values for 6 months. After patients had received CyA treatment for 1 month, the total power of systolic blood pressure variability and its low-frequency component remained higher than before treatment ($P < 0.05$). At 3 and 6 months, heart rate was decreased ($P < 0.001$) when high-frequency power of RR variability and plasma atrial natriuretic peptide were increased ($P < 0.05$). As plasma noradrenaline never exceeded the baseline range, the hypertensive effect of CyA was not likely to have resulted from sympathetic activation. Rather, the increase in systolic blood pressure variability, its low-frequency component and the pattern of pulse pressure changes, pointed to a direct vascular effect of CyA. The maintenance of a normal cardiac baroreflex function during 6 months of CyA treatment might be pivotal to the adaptive responses towards direct CyA effects.

Keywords Atrial natriuretic peptide · Baroreflex · Cyclosporine · Heart rate and blood pressure variability · Noradrenaline · Pulse pressure

Introduction

Cyclosporine (CyA), one of the most effective immunosuppressive agents available, prolongs survival after organ transplantation but is also associated with a number of serious side effects. In addition to inhibiting T-cell activation [1], CyA causes hypertension and renal dysfunction [2]. How CyA treatment results in arterial hypertension remains unclear. An enhanced sympathetic activity has been hypothesised, with consequent vasoconstriction [3, 4], activation of neurohormonal vasoconstrictors [5, 6] and alterations in vascular sensitivity to

natural vasoactive mediators [7]. Several studies in animals [5, 8] and in humans [4] reported increased plasma noradrenaline parallel to increased arterial blood pressure after several months or years of CyA treatment. Conversely, Stein et al. reported CyA-impaired peripheral vasodilatation without increased sympathetic activity [9].

In vitro studies provide evidence of a direct vasoconstrictive effect of CyA on human vessels [7, 10]. In vivo, this effect might trigger feedback control and, in turn, decrease the physiological vasoconstrictive tone. Evidence has been found that the baroreflex circulatory control is maintained under CyA treatment [11]. A few short-term studies have described the effects of CyA in healthy subjects [12]. Most human studies have focused on effects in patients already treated for months or years, after having benefited from organ grafts. Most severe and prolonged diseases that lead to grafting of the heart, kidney or liver, cause long-lasting metabolic impairments that are harmful to the nervous system. These diseases also cause profound shifts in the balance of the hormonal and neural controls. It is often difficult for one to determine whether some abnormal setting of the neuro-endocrine control system reflects the consequences of the original disease, the response of the control system to an impaired organ function, the strain of a side effect, or whether it is a combination of these conditions.

We attempted to differentiate the direct cardiovascular effects of CyA and the ensuing adjustments of autonomic control through serially repeated testing of patients who were entitled to receive CyA treatment because of a high rejection risk following corneal transplant. In such subjects, no severe organ disease had impaired the autonomic nervous system or shifted the neuro-endocrine controls involved in the regulation of arterial blood pressure. Changes in spontaneous baroreflex, arterial blood pressure, heart rate, and neural and endocrine indicators of the control of circulatory functions, were repeatedly assessed in these subjects. This was carried out through sequential testing, which we performed before CyA treatment, 1 week after beginning the CyA treatment and after CyA intake for 1, 3 and 6 months.

Subjects and methods

Patients

Eight patients (seven men, one woman) aged 44 to 76 years (mean age 62.6 years) were studied (Table 1). The study was approved by our institutional ethics committee, and informed consent was obtained from each patient. Patients had no previous history of cardiovascular, neurological, kidney or liver disease. All were in good general condition, as judged from the pre-anaesthetic medical examination and biological survey. They received no vasoactive treatment or any medication known to interact with the activity of the autonomic nervous system. Various disease conditions led to the proposal of the corneal graft, and CyA (Neoral) was prescribed because of a high rejection risk of the graft (Table 1). In six subjects previous grafting attempts, carried out without immunosuppressive treatment, had failed. Patients began oral CyA treatment the day before surgical grafting and were assessed 1 week before, 1 week after receiving the graft and then 1, 3 and 6 months after treatment. On the day of the grafting procedure, and on the following day, patients received 125 mg/day methylprednisolone, intravenously. The patients left hospital 1 week after the graft and were then assessed, on a monthly basis, for the outcome of the grafted eye and their general condition. Whole-blood CyA concentration was monitored every other day during the first week, and then every 2 or 3 weeks to allow for regular adjustment of intake dosage, with a targeted whole-blood concentration in the range of 150–200 ng/ml.

General organisation of the study

Patients were instructed to avoid cigarettes, alcohol, or beverages that contained caffeine, for 12 h, as well as

Table 1 Anthropometric characteristics and ophthalmic conditions of the subjects (*M* male, *F* female)

Patient No.	Age	Gender	Height (m)	Weight (kg)	Initial diagnosis	No. of graft attempts	Eye outcome after 6 months CyA
1	68	M	1.72	71	Traumatic keratitis	2nd	Clear graft/good visual acuity
2	57	M	1.7	95	Secondary endothelial dystrophy	3rd	Rejection after 5 months
3	45	M	1.69	80	Herpes keratitis and neovascularisation	2nd	Clear graft/good visual acuity
4	76	M	1.75	67	Deep corneal neovascularisation	1st	Clear graft/good visual acuity
5	61	M	1.61	67	Deep corneal neovascularisation and herpes keratitis	1st	Clear graft/good visual acuity
6	69	M	1.64	73	Deep corneal neovascularisation	3rd	Clear graft/good visual acuity
7	76	F	1.65	47	Secondary endothelial dystrophy	3rd	Clear graft/good visual acuity
8	44	M	1.79	80	Bacterial keratitis/deep corneal neovascularisation	2nd	Rejection after 4 months

strenuous exercise for 24 h, before each test session. Functional testing was completed at least 2 h after the patients had consumed a light breakfast in a quiet, dimly lit room, at a temperature of 22–24°C. The patient rested at first in a supine position for 20 min after having been connected to an electrocardiograph and was asked to remain as relaxed as possible. Breathing rate was assessed in all patients (measured against a metronome), and it was checked that no variation greater than 3 cycles/min occurred in any subject between the different sessions. After an intravenous catheter had been inserted in the forearm, the subject rested for another 10 min before venous blood was sampled. A photoplethysmographic sensor for blood pressure (BP) (Finapres, Ohmeda, Englewood, Co., USA) was placed on the third finger of the right hand and, from then on, was kept at heart level during the whole measurement sequence. Data were recorded while the patient rested in a supine position for 10 min and then after the patient had been tilted upright at 60° for 10 min. A second blood sample was taken at the end of the 10 min upright position.

Collection of values of RR intervals and blood pressure

The RR intervals were measured after we had processed the QRS complexes from an electrocardiograph with a peak detection circuit. Finger systolic blood pressure (SBP), diastolic blood pressure (DBP), and RR intervals collected for each cardiac cycle were stored on a microcomputer via an analogue-to-digital converter (Metrabyte DAS-16) for analysis later.

Data analysis

The haemodynamic status and autonomic nervous system activity before and during CyA treatment were assessed through several indices:

- Values of SBP, DBP, pulse pressure (PP) and heart rate (HR). These were averaged from beat-by-beat measurements over an 8 min steady state in the supine position and an 8 min steady state in the upright position.
- Baroreflex sensitivity. This was assessed by the sequence method. The HR response to BP variations is reflected in sequences of three or more beats in which the SBP and the following RR-interval changes in the same direction (either increasing or decreasing) [13]. Such spontaneous baroreflex events were computer-searched and counted, and a linear regression was calculated for all the sequences detected during one steady-state period. The slope of this regression was taken as a quantitative index of spontaneous baroreflexsensitivity (ms/mmHg) [13].
- Spectral analysis of heart rate variability (HRV) and blood pressure variability (BPV). We filtered the RR-interval time series to discard the small number of abnormal RR intervals caused by incorrect triggering by the T-wave, missing R-wave or an occasional premature beat. HRV and BPV were evaluated by coarse graining spectral analysis (CGSA) [14]. This method accounts for simultaneous harmonic oscillations as well as non-harmonic or fractal variations in spontaneous HRV and BPV. For HRV and BPV we analysed the harmonic spectrum components to determine the power in the domains of low frequencies (LFs) (0.04–0.15 Hz) and high frequencies (HFs) (0.15–0.50 Hz). For HRV, these components of spectral power were considered separately. HF power and the normalised indicator, HF/total spectral power (tot), were used as indicators of the activity of the parasympathetic system. LF/tot and the ratio LF/HF were considered as indicators of the activity of the sympathetic nervous system [14]. In BPV, the LF region is mediated by sympathetic modulation of the vascular tree and other neurohumoral mechanisms [14].
- Neurohormonal mediators. In each blood sample the plasma concentrations of noradrenaline, adrenaline and dopamine were determined by a sensitive and specific radio-enzymatic method [15]. We used commercially available radioimmunoassays to measure plasma aldosterone, active renin, endothelin and atrial natriuretic peptide (ANP). In the blood, nitric oxide (NO) is rapidly inactivated by interaction with haemoglobin or is oxidised to form different nitrogen oxides (NOx), mainly nitrate (NO_3) which provides an index of the endogenous formation of NO [16]. The NO_3 quantities were measured through chemiluminescence counting (NO analyser; NOA 280, Sievers Instruments, Boulder, Co., USA).

Statistical analysis

Data are presented as means ± SEM. Changes in steady values of BP and HR were evaluated by analysis of variance (ANOVA) for repeated measurements (numerous serial measures in the same position). Due to the small number of subjects, Wilcoxon's signed rank test was used for paired comparisons, i.e. values at different dates with baseline period. A P value of less than 0.05 was considered to be statistically significant.

Results

Six months after grafting had taken place, the ocular outcome was good in six patients (Table 1). Failure of the graft occurred after 4 and 5 months in two patients

in whom CyA treatment was then discontinued. Cardiovascular testing after 6 months was performed in the six patients that were still being treated. The average (± SEM) blood concentrations of CyA were 124 ± 13 ng/ml after 1 week, 201 ± 34 ng/ml after 1 month, 195 ± 37 ng/ml after 3 months, and 191 ± 20 ng/ml after 6 months. Plasma creatinine was in the normal range for every subject (62–105 μmol/l) and did not increase significantly during the CyA treatment (69–112 μmol/l).

The subjects did not complain of disturbing symptoms, and the changes in BP described hereafter were clinically well tolerated. Apart from topical eye medications, no treatment other than CyA was used and, in particular, no anti-hypertensive medication was prescribed.

Arterial blood pressure and heart rate

SBP and DBP were significantly increased above baseline levels when the patient was in both the supine and upright positions after 1 week, and after 1, 3 and 6 months of CyA intake (ANOVA, $P < 0.001$; Table 2;

Table 2 Spectral analysis of BP and HRV, and indices of spontaneous baroreflex in the supine and upright position. Values are means ± SEM

Parameter	Baseline	With cyclosporine treatment			
		1 Week	1 Month	3 Months	6 Months
SBP (mmHg)					
Supine	128.5 ± 1.7	142.5 ± 3.1[a]	157.3 ± 4.4[a]	148.4 ± 4.9[a]	151.6 ± 5.1[a]
Tilt 60°	132.7 ± 2.1[b]	143.9 ± 3.8[a]	160.3 ± 2.9[a,b]	141.6 ± 3.4[a,b]	143.5 ± 7.2[a,b]
PtotSBP ($mmHg^2$)					
Supine	11.4 ± 2.2	23.6 ± 13.0	52.6 ± 17.4[a]	48.0 ± 12.5[a]	31.1 ± 7.7[a]
Tilt 60°	23.6 ± 11.4	24.0 ± 8.1	27.3 ± 4.8	35.1 ± 10.3	46.0 ± 8.6
LFSBP ($mmHg^2$)					
Supine	3.2 ± 1.2	12.7 ± 8.4	27.8 ± 14.9[a]	21.7 ± 4.4[a]	10.4 ± 2.1[a]
Tilt 60°	4.5 ± 2.0[b]	6.5 ± 1.8	8.2 ± 3.0	12.3 ± 5.0	10.9 ± 5.5
DBP (mmHg)					
Supine	68.4 ± 0.9	75.9 ± 0.2[a]	89.3 ± 1.3[a]	77.6 ± 1.8[a]	84.2 ± 3.0[a]
Tilt 60°	77.1 ± 1.1[b]	82.9 ± 1.7[a,b]	94.5 ± 2.0[a,b]	80.3 ± 1.8[a,b]	87.7 ± 4.6[a,b]
PtotDBP ($mmHg^2$)					
Supine	3.1 ± 0.9	5.5 ± 1.6	14.3 ± 4.8[a]	11.5 ± 3.1[a]	6.9 ± 0.5
Tilt 60°	6.9 ± 2.0[b]	12.3 ± 5.0	8.3 ± 3.3	8.8 ± 2.6[a]	62.4 ± 45.0
LFDBP ($mmHg^2$)					
Supine	1.9 ± 0.7	3.5 ± 1.0	9.1 ± 4.5[a]	4.9 ± 1.5[a]	4.5 ± 0.7
Tilt 60°	3.8 ± 1.1	4.9 ± 1.6	3.8 ± 1.5	4.0 ± 1.7	23.6 ± 15.3[a]
PP (mmHg)					
Supine	57.6 ± 2.3	62.7 ± 2.3[a]	86.4 ± 6.3[a]	75.3 ± 4.5[a]	67.5 ± 3.9[a]
Tilt 60°	54.8 ± 2.4[b]	52.7 ± 2.9[a,b]	83.2 ± 4.9[a,b]	63.6 ± 4.0[a,b]	55.7 ± 4.6[b]
RR (ms)					
Supine	882 ± 10	859 ± 10[a]	887 ± 15	963 ± 15[a]	937 ± 19[a]
Tilt 60°	776 ± 9[b]	735 ± 14[a,b]	755 ± 9[a,b]	798 ± 10[a,b]	798 ± 15[a,b]
PtotRR (ms^2)					
Supine	904 ± 436	646 ± 259	1230 ± 603	1001 ± 428	1678 ± 716
Tilt 60°	365 ± 168[b]	553 ± 396	349 ± 238	242 ± 92	541 ± 61
HFRR (ms^2)					
Supine	77.8 ± 51.6	36.6 ± 18.6[a]	87.3 ± 42.4	144.9 ± 76.8[a]	176.3 ± 78.0[a]
Tilt 60°	16.4 ± 8.1[b]	39.0 ± 37.9	6.2 ± 3.7[b]	6.6 ± 4.5[b]	58.8 ± 22.7
HF/tot					
Supine	0.11 ± 0.05	0.06 ± 0.01[a]	0.07 ± 0.03	0.12 ± 0.03	0.14 ± 0.06
Tilt 60°	0.05 ± 0.02[b]	0.03 ± 0.02	0.02 ± 0.01[a]	0.02 ± 0.01[a,b]	0.1 ± 0.03
LFRR (ms^2)					
Supine	160 ± 83	232 ± 122	710 ± 410	495 ± 272	860 ± 405
Tilt 60°	103 ± 61	71 ± 38[b]	85 ± 67[b]	51 ± 25	155 ± 32
LF/tot					
Supine	0.18 ± 0.08	0.24 ± 0.08	0.39 ± 0.11	0.36 ± 0.09[a]	0.41 ± 0.07[a]
Tilt 60°	0.22 ± 0.06	0.17 ± 0.06	0.16 ± 0.04	0.17 ± 0.05[b]	0.28 ± 0.03
LF/HF					
Supine	5.55 ± 3.01	7.78 ± 3.64	33.29 ± 25.27	4.73 ± 2.27	7.90 ± 4.05
Tilt 60°	16.68 ± 15.05	4.27 ± 1.98	13.74 ± 8.63	11.23 ± 5.11	175.61 ± 134.44
Number of spontaneous baroreflex sequences					
Supine	25.8 ± 8.5	18.4 ± 5.5[a]	37.0 ± 6.1	29.5 ± 9.4	30.0 ± 11.3
Tilt 60°	34.0 ± 8.3[b]	20.5 ± 8.3[a,b]	22.8 ± 11.0	29.6 ± 12.5	31.5 ± 10.6
Baroreflex slope (ms/mmHg)					
Supine	11.6 ± 2.4	11.9 ± 3.5	10.5 ± 2.6	14.9 ± 3.9	15.9 ± 2.6
Tilt 60°	6.4 ± 1.2[b]	5.8 ± 1.3[b]	5.5 ± 1.1[b]	6.7 ± 1.5[b]	6.3 ± 1.6[b]

[a] $P < 0.05$ vs before treatment (treatment effect)
[b] $P < 0.05$ vs supine (posture effect)

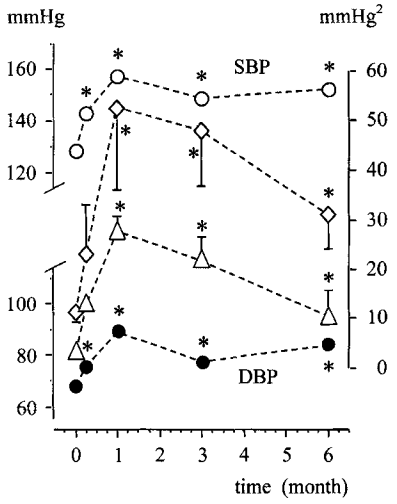

Fig. 1 Serial changes in mean values of arterial blood pressures and the spectral power (total and LF component) of systolic arterial pressure variability with the patient in the supine posture. *Circles* arterial pressure, *diamonds* total power of SBP variability, *triangles* low-frequency component of SBP. On the time axis: *0* baseline testing before CyA treatment and corneal graft. $*P < 0.05$ compared with baseline

Fig. 1). PP increased over baseline in either posture after 1 week of CyA, and remained so during 3 months of treatment (ANOVA, $P < 0.001$; Table 2). After 6 months of treatment, supine PP was still higher than baseline ($P < 0.001$) but upright PP was not different from baseline. In either posture, HR was higher than baseline after 1 week, identical to baseline after 1 month, and significantly lower after 3 and 6 months (Table 2; Fig. 2).

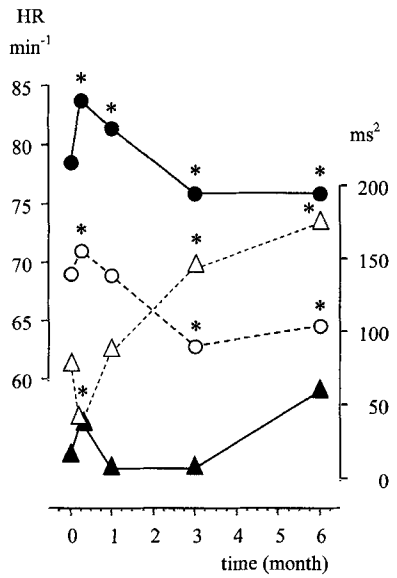

Fig. 2 Serial changes in mean values of HR and the spectral power of the HF component of RR variability. *Circles* heart rate (left axis), *triangles* HF component (right axis), *open symbols* and *broken lines*: supine, *solid symbols* and *continuous lines* 60° upright values. On the time axis: *0* baseline testing before CyA treatment and corneal graft. * $P < 0.05$ as compared to baseline

Spontaneous baroreflex and spectral analysis of BP and HR variability

The number of spontaneous baroreflex sequences was reduced whatever the recording position after the first week of CyA intake, and thereafter resumed baseline values (Table 2). No significant change occurred in the baroreflex slope throughout the study, in the supine or upright position. The physiological decrease in baroreflex slope when the patient was changed from supine to upright position was found at each testing period (Table 2).

The total spectral power of the SBP variability and LF_{SBP} component during supine rest were increased significantly after 1, 3 and 6 months of CyA treatment ($P < 0.04$, Table 2; Fig. 1), but no significant change occurred serially in the values measured in the upright posture. The significant increase in LF_{SAP} when the patient was tilted upright was present at baseline but not upon CyA treatment (Table 2). Both the total power and the LF_{DBP} component of variability were increased significantly during supine rest after 1 month ($P < 0.04$; Table 2).

The decrease in the total power of RR variability due to upright tilting was significant at baseline but not upon CyA treatment (Table 2). With the patient in the supine posture the HF_{RR} component was significantly decreased after 1 week of CyA treatment ($P < 0.05$; Table 2; Fig. 2), had increased slightly after 1 month and was significantly higher than baseline after 3 and 6 months of CyA intake ($P < 0.05$; Table 2; Fig. 2). The HF_{RR} decrease with upright tilting was significant before treatment and also after 1 and 3 months of CyA intake ($P < 0.05$; Table 2). In neither posture did the LF_{RR} component change significantly during CyA treatment, but an increase in the LF/tot index occurred after 3 and 6 months of CyA treatment during supine rest (Table 2). No significant change was seen in the LF/HF index.

Humoral variables

In all patients the plasma concentration of noradrenaline was significantly reduced 1 week after CyA treatment in both the supine and upright positions (supine 196 ± 37 vs 315 ± 45 ng/ml at baseline; upright 324 ± 71 vs 503 ± 49 ng/ml at baseline; $P < 0.01$; Table 3; Fig. 3). After CyA treatment, at 1, 3 and 6 months, plasma noradrenaline values were not significantly different from baseline. No significant change had occurred in plasma concentrations of adrenaline, dopamine, aldosterone or active renin (Table 3). No significant change had occurred in plasma endothelin and plasma nitrates (Table 3). Plasma ANP was significantly increased in either posture after 1, 3 and 6 months of CyA treatment (Table 3).

Table 3 Plasma concentrations of neuro-humoral mediators. Values are means ± SEM. *AVP* arginine vasopressin or antidiuretic hormone

Neuro-humoral mediator	Baseline	With cyclosporine treatment			
		1 Week	1 Month	3 Months	6 Months
Noradrenaline (pg/ml)					
Supine	315 ± 45	196 ± 37[a]	271 ± 62	288 ± 61	335 ± 88
Tilt 60°	503 ± 49[b]	324 ± 71[a,b]	522 ± 80[b]	517 ± 88[b]	583 ± 116[b]
Adrenaline (pg/ml)					
Supine	45.5 ± 10.9	36.6 ± 6.1	35.2 ± 7.5	40.0 ± 4.4	33.0 ± 1.5
Tilt 60°	47.6 ± 7.2	45.6 ± 11.6	62.2 ± 5.1[a]	45.4 ± 6.8	42.3 ± 3.1
Dopamine (pg/ml)					
Supine	57.7 ± 8.8	62.8 ± 9.0	64.8 ± 11.5	61.3 ± 11.7	50.7 ± 6.4
Tilt 60°	62.7 ± 5.2	59.2 ± 9.3	73.1 ± 11.3[b]	62.5 ± 9.6	46.0 ± 5.3[a]
AVP (pg/ml)					
Supine	0.65 ± 0.22	0.58 ± 0.2	0.86 ± 0.2	1.2 ± 0.3	0.7 ± 0.2
Tilt 60°	1.25 ± 0.38	1.35 ± 0.35	1.22 ± 0.25	1.6 ± 0.42	1 ± 0.32
Active renin (pg/ml)					
Supine	4.50 ± 1.01	3.83 ± 1.00	4.00 ± 0.71	4.20 ± 0.86	4.67 ± 0.26
Tilt 60°	5.17 ± 1.04	4.00 ± 1.13	4.40 ± 0.98	5.40 ± 1.25	4.33 ± 0.68
Aldosterone (pg/ml)					
Supine	83.8 ± 15.2	95.2 ± 11.3	95.3 ± 24.5	72.6 ± 15.0	112.2 ± 28.3
Tilt 60°	92.4 ± 9.7	122.7 ± 16.0	105.5 ± 14.5	102.3 ± 19.2[b]	115.7 ± 28.5
Endothelin (fmol/ml)					
Supine	7.6 ± 0.7	7.4 ± 0.8	7.9 ± 1.1	9.3 ± 1.6	11.7 ± 0.9[a]
Tilt 60°	7.8 ± 0.7	7.8 ± 1.2	7.8 ± 1.1	9.3 ± 1.1	10.3 ± 1.4
Nitrate (μmol/l)					
Supine	29.7 ± 10.5	26.3 ± 2.7	34.3 ± 6.0	32.2 ± 4.2	29.3 ± 3.7
Tilt 60°	30.5 ± 11	26.8 ± 2.4	30.4 ± 5.1	31.6 ± 4.1	29.2 ± 3.9
ANP (pg/ml)					
Supine	14.6 ± 5.7	27.1 ± 15.1	23.2 ± 9.5[a]	68.0 ± 35.7[a]	64.2 ± 21.5[a]
Tilt 60°	21.5 ± 9.4	26.5 ± 15.2	35.0 ± 15.1[a]	36.9 ± 18.7[a]	70.8 ± 31.9[a]

[a] $P < 0.05$ vs before treatment (treatment effect)
[b] $P < 0.05$ vs supine (posture effect)

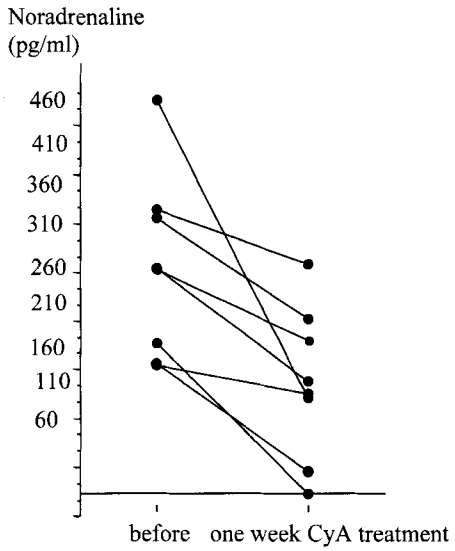

Fig. 3 Individual values of supine plasma noradrenaline before and after 1 week of CyA treatment. In all patients, plasma noradrenaline was reduced after 1 week of CyA treatment

Discussion

A sequence of cardiovascular effects related to CyA intake was observed in patients free of long-term metabolic impairments and receiving no medication that was likely to affect neuro-endocrine controls. Firstly, the early increase in arterial BP was paralleled by a transient reduction of plasma noradrenaline, which resumed normal values within 1 month and remained in the normal range during the following 5 months of CyA intake. Secondly, even though during 6 months the arterial BP and its variability remained higher than baseline, after 1 month there was normal baroreflex sensitivity and an increased release of atrial natriuretic peptide, which probably prompted the coping of cardiovascular activities with the direct effect of CyA on arterial smooth muscle.

Arterial blood pressure and vasomotor tone

In a resting condition plasma noradrenaline is well correlated to muscle sympathetic nerve activity [17] and to DBP [18]. The fact that plasma noradrenaline never exceeded the baseline range during the 6 months indicated that CyA treatment was hardly consistent with increased activity of the sympathetic nervous system bearing the CyA-related hypertension. This observation was in agreement with previous evidence that CyA did not change noradrenaline spillover and muscle sympathetic discharges [9, 19]. Conversely, the transient decrease in plasma noradrenaline after the first week of treatment would be consistent with a baroreflex-mediated limitation of the efferent sympathetic tone, as

triggered by the CyA-related increase in arterial pressure. A similar decrease in plasma noradrenaline has been also observed in healthy subjects after acute inhibition of nitric oxide synthesis [20]. The further increase in arterial BP from 1 week to 1 month of treatment was paralleled by the resumption of plasma noradrenaline towards baseline values. The pattern of changes in PP showed the serial changes in arterial tone. In patients with hypertension the increased PP is linked to the increased stiffness of the arterial wall, leading in turn to a faster reflection of the pressure wave [21]. In our study supine PP increased after 1 week of CyA treatment at a time when the level of plasma noradrenaline was markedly lowered. After 1 month of treatment, PP further increased both in the supine and upright positions, when noradrenaline resumed normal values. Thus, these changes appeared to outline the pharmacological influences that separately or together acted on the arterial walls of the patients. Sympathetic vasomotor control was further assessed by spectral analysis of BP variability. The total spectral power of SBP variability and its LF_{SBP} component are considered as oscillatory markers of sympathetic vasomotor modulation [14]; during 1 to 6 months of CyA treatment, these indices were significantly increased in the supine position. Since plasma noradrenaline did not exceed baseline level, and the indices of cardiac sympathetic modulation (LF_{RR} component and LF/HF index) also remained unchanged, the increase of LF_{SBP} was unlikely to have been triggered by a genuine increase in sympathetic activation. The LF_{SBP} increase would then reflect a direct effect of CyA to increase the arterial vasomotor tone. Indeed, a direct vasoconstrictor effect, mediated through α-receptors, has been reported [22, 23]. Together with another vasoconstrictive, apparently calcium-dependent, action [10], this effect causes hypertension by altering vascular reactivity. A greater LF_{SBP} component has been found, in both the supine and upright positions, in patients with essential hypertension [24]. The treatment of our initially normotensive patients with CyA appeared to alter BP variability, perhaps in a fashion similar to that which occurs in essential hypertension. In the study by Castellano et al. [20] nitric oxide inhibition hindered the vasodilating tone and triggered, in turn, both the baroreflex limitation of plasma noradrenaline and the lowering of LF_{SBP}. However, in our study the vascular effects of CyA treatment over 6 months were neither nitric oxide nor endothelin dependent, at least as reflected by the plasma levels of these mediators. Other short-term studies of CyA effects also found no evidence to implicate circulating endothelin [12] or nitric oxide in acute changes of cardiovascular function [25]. Several studies supported the notion that CyA might affect functional vessel properties, i.e. through decreasing arterial compliance [24] and vascular reactivity to several agonists [9, 26]. In our study, the addition of the effects of CyA, per se, to the apparently normal noradrenergic release, as judged from plasma levels, resulted in an increased LF_{SBP} component. A study by Lucini et al. [11, 24] on the effects of CyA did not find the increased LF_{SBP} that we observed. We believe that at least three elements may contribute to this discrepancy. Firstly, in the study by Lucini et al. the patients treated with CyA had received heart or solid-organ transplant and were, therefore, more likely to have impaired or shifted autonomic control than our patients. Secondly, whereas our patients did not receive antihypertensive treatment, the patients of Lucini et al. were given calcium blockers, which probably prevented some consequences of the direct effects of CyA. Thirdly, our testing was performed earlier in the course of CyA treatment, and fixed alterations in the vessel walls, including baro-receptor areas, are more likely to develop after long-term treatment than after a few months.

Heart rate and baroreflex

We observed a transient HR increase after 1 week of CyA treatment. An increase in HR has also been reported to parallel a transient fourfold increase in plasma CyA occurring 2 h after the usual intake in kidney transplant recipients [27]. The transient HR increase after 1 week of CyA treatment was consistent with the concomitantly lowered HF_{RR} component reflecting the decreased vagal influence on cardiac activity in the supine posture. Such a dampened vagal tone was paradoxical at first, since the expected baroreflex response to a sudden increase in arterial pressure is a rise in vagal tone, in turn lowering the HR. However, in the bronchial wall, for example, the parasympathetic tone is physiologically down-modulated through sympathetic activation. The sympathetic dampening of vagal control of HR has also been suspected during hypertension [28]. With CyA, the direct effect already thought to operate in the vascular wall might also hinder parasympathetic heart control. The condition we observed was transient 1 month after the beginning of treatment, both HR and its HF_{RR} component had resumed baseline values, and, after 3 and 6 months of treatment, HR values decreased significantly below baseline while HF_{RR} power reached twice the baseline levels. The increase in plasma ANP developed parallel to the other indicators of an increased parasympathetic tone [29] and probably contributed to the opposing of the increased vascular tone [30]. Six months after CyA treatment, the baroreflex slope value was unchanged when compared with baseline. Thus, in accordance with previous results [11], the autonomic modulation of cardiac activity was not impaired, and, after 6 months of CyA treatment, the baroreflex responses to the CyA-induced hypertension were

maintained. On the whole, our findings show that in these subjects, 6 months of treatment with CyA did not conspicuously alter the baroreflex control of HR, which further argues for the notion that CyA causes hypertension through impairment of vascular mechanics.

In conclusion, we studied subjects receiving cyclosporine after corneal transplantation, i.e. patients whose neural control mechanisms did not support the consequences of severely altered metabolic functions as occurs in patients who receive cardiac, kidney, or liver transplantation. The subjects were serially examined before and after they had received CyA for 1 week and 1, 3 and 6 months. Evidence was obtained that 6 months of exposure to CyA did not conspicuously alter the baroreflex control of cardiovascular functions. The hypertensive effect of CyA treatment decreased arterial compliance, as reflected in PP changes, but was not borne by activation of the sympathetic nervous system. The marked increase in BPV, without a significant increase in plasma noradrenaline, further suggested that CyA might induce hypertension through direct modulation of smooth muscle tone, at least during the first 6 months of treatment. The significant decrease of HR to below baseline after 3 and 6 months suggested that cardiac autonomic modulation was preserved. All in all, the transient decrease in plasma noradrenaline, the secondary lowering of HR paralleled by an increased HF_{RR}component, and an increasing plasma ANP, all appeared as instrumental for coping with a direct vasoconstrictive side effect of CyA.

Limitations of the study

The interpretation of the components of HR and BPV is complex and a matter of debate. Nevertheless, this approach provides indices of the autonomic modulation of the sinus node and the vasculature that has attracted widespread international consensus [31, 32].

The number of subjects remained limited. The need for one to propose CyA treatment for corneal graft is not frequent, and not all patients fulfilled the criteria to be entered into the study: patients with mild degrees of cardiovascular or metabolic disease are not infrequent. In addition, some patients who might have joined the study were hindered by the need to undergo the serially repeated test sessions. It should be noticed, however, that significant statistical results were obtained (regarding cardiovascular variables, plasma concentrations of noradrenaline and atrial natriuretic peptide, and indices of HR and BPV) because all the subjects had similar serial profiles, i.e. the changes reflected similar directions of responses.

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