REGULAR ARTICLE

The Peripheral Serotonergic System and Platelet Aggregation in Cyclosporin A-Induced Hypertensive Rats

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Abstract

Cyclosporin A plays an important role in preventing rejection in allograft transplant recipients. However, the therapeutic use of cyclosporin A is associated with increased incidence of thromboembolic complications and drug-related hypertension. In order to study the mechanisms by which cyclosporin A induces these abnormal pathophysiological situations, we have assessed the platelet serotonin contents and whole blood platelet aggregation in control rats as well as in rats treated (orally) with 30 and 5 mg/kg/day of cyclosporin A, after 2 and 7 weeks of treatment. These doses correspond respectively to CsA "peak" and "trough" concentrations achieved in human blood in clinical practice (immediately following an intake of a daily dose of CsA and when the blood concentration stabilizes, respectively). Both trough and peak doses caused an increase in blood pressure after 2 and 7 weeks. Platelet serotonin content decreased in the cyclosporin-treated groups, in contrast with the control. Collagen-induced whole blood platelet aggregation increased drastically for the peak concentration-treated group, while adenosine 5′-diphosphate-induced platelet aggregation did not reach statistical significance. Finally, in vitro platelet thromboxane A_2 generation increased in cyclosporin A concentrations when platelets were stimulated with either collagen or adenosine 5′-diphosphate. In conclusion, both tested cyclosporin A concentrations induced important changes in platelet serotonin and thromboxane content and aggregation, factors which may play a decisive role in the development and/or maintenance of hypertension and thrombotic complications. © 1999 Elsevier Science Ltd. All rights reserved.

Cyclosporin A (CsA) is a potent and clinically relevant immunosuppressive agent and is widely used in organ transplantation [1,2] and also in the treatment of autoimmune diseases [3]. However, despite its effectiveness, CsA therapy has been associated with drug-related hypertension [4–7] and increased risk of thromboembolic complications [8–10], which may be partly due to the direct effects of CsA on platelets [11–14].

It is well known that platelets may play an important role in the pathophysiology of hypertension and also in the pathogenesis of thrombosis. Activated platelets are a source of the most potent,

naturally found vasoconstrictors, such as thromboxanes and endoperoxides. Many studies have reported cyclosporin A-induced changes in the thromboxane/endoperoxides balance [13,15,16], in enhanced hemostasis and reduced thrombolysis [17,18], in increased platelet aggregability [12–14,19], as well as in altered peripheral serotonergic mechanisms [14,20–22]. Serotonin (5-hydroxytryptamine, 5-HT), mainly present in platelets, has been linked with thrombotic complication and increase in blood pressure [23,24]. When activated, platelets adhere and aggregate to the damaged endothelium, releasing (among other compounds) serotonin and thromboxane A2 (TXA_2), which may act as vasoconstrictors and also extend aggregation to other agonists, thus promoting thrombus formation.

Therefore, the purpose of this study was to determine the effects of CsA on the peripheral serotonergic system, platelet aggregation, and TXA_2 generation.

1. Materials and Methods

1.1. Animals and Diets

Male Wistar rats (Charles River Laboratories Inc., Barcelona, Spain), ~300 g, were maintained in an air-conditioned room, subjected to 12-hour dark/light cycles and given standard laboratory rat chow and free access to tap water. Animal experiments were conducted according to the European Convention on Animal Care, and the whole research project was approved by the Portuguese National Foundation for Science and Technology. The rats were divided into three groups: one group received orange juice only (control) and the others received 5 and 30 mg/kg/day of CsA (Sandimmun Neoral, Sandoz Pharma, Basel, Switzerland) dissolved in orange juice for 7 weeks. Blood pressure values (systolic, diastolic, and mean) were measured using a tail-cuff sphygmomanometer. Thromboxane in vitro studies were carried out through the incubation of platelets with CsA (dissolved in dimethyl sulfoxide) at 0.1 and 1.0 µmol/L concentrations. These concentrations were calculated to match the real blood concentrations of patients taking CsA: 1.0 µmol/L immediately after an intake of a daily dose of CsA ("peak") and 0.1 µmol/L, when the blood concentration stabilizes ("trough"). These concentrations also corresponded to the 30 and 5 mg/kg/day of CsA administered to the rats.

1.2. Collecting the Blood and Preparing the Platelets

Following intraperitoneal ketamine anesthesia, blood was withdrawn by venipuncture from the jugular vein and added to an anticoagulant solution (0.1 mL/mL blood) containing (in mmol/L) citric acid (71), sodium citrate (85), and D-glucose (111). The blood was centrifuged ($160 \times g$ for 10 minutes at 20°C) to obtain platelet-rich plasma (PRP) and the platelets were then recovered by recentrifugation at $730 \times g$ for 10 minutes at 20°C.

1.3. Platelet Serotonin Contents

The platelet pellet was resuspended in 1 mL of a buffer solution (pH 7.4) containing (in mmol/L): NaCl (145), KCl (5), $MgSO_4$ (1), $CaCl_2$ (1), D-glucose (10), and 120 µL of perchloric acid (70%) was added. Following 15 minutes at ice temperature, the suspension was finally centrifuged at $730 \times g$ for 10 minutes at 20°C and the supernatant containing the released serotonin was collected for quantification. Platelet 5-HT was determined by high pressure liquid chromatography with electrochemical detection. The chromatographic system consisted of a Gilson Applied Chromatographic System (Middleton, WI, USA) with a 305 model pump and a 231 injection valve model, with a 50-µL loop. A Biophase ODS Reverse Phase 18 analytical column (250×4.6, $\varnothing = 5$ µ, Bioanalytical Systems Inc., West Lafayette, IN, USA) was used and separation was made possible by using an isocratic solvent system consisting of an acetate-citrate buffer (sodium acetate 0.1 mol/L, citric acid 0.1 mol/L) containing sodium octane sulphonate (0.5 mmol/L), etylenedinitrilo-tetraacetic acid (0.15 mmol/L), dibutylamin (1 mmol/L), and 10% methanol. A flow rate of 1 mL/minute was maintained and detection of the chromatographed serotonin was achieved by using a 141 Gilson electrochemical detector model (650 mV).

1.4. Whole Blood Platelet Aggregation

Whole blood platelet aggregation was monitored by measuring electric impedance, using a Chrono-

log aggregometer (Chrono Log, Havertown, PA, USA). This technique is based on the detection of changes in electrical resistance between two electrodes submerged in the sample. Fresh heparinized (0.5 mL) whole blood and 0.9% NaCl (0.5 mL) were mixed using a magnetic stirrer and allowed to balance at 37°C for 5 minutes before adding the agonists adenosine 5′-diphosphate (1.0 μmol/L) and collagen (5 μg/mL). Platelet count and mean platelet volume were measured with a Coulter counter.

1.5. Platelet Thromboxane B_2 Generation

The blood was collected into plastic syringes containing 1 vol of 3.8% trisodium citrate for every 9 vol of blood. Aliquots were incubated for 3 hours at 37°C with either solvent (control) or CsA in final concentrations of 0.1 and 1.0 μmol/L. The remaining blood was divided into aliquots containing CsA or solvent to measure the potentially maximum amount of releasable thromboxane. After incubation, whole blood samples were centrifuged as described previously to obtain PRP and platelet-poor plasma (PPP). Platelets were counted using the Coulter counter (350,000±50,000 platelets/μL). They were subsequently activated with 1.0 μmol/L of ADP and 5 μg/mL of collagen. Following aggregation, the supernatants were assessed to determine the amount of thromboxane B_2 [(TXB_2) the stable end product of TXA_2] generated. Briefly, the contents of the cuvette was transferred to a microcentrifuge tube containing 10 μmol/L etylene-dinitrilo-tetraacetic acid and 10 μmol/L indomethacin and centrifuged at 12,000×g for 4 minutes. The resulting supernatant was frozen at −70°C to later analyse the TXB_2. A sample of PRP merely spun without the addition of ADP or collagen was also assessed to serve as a control for any spontaneous TXB_2 generation. To determine the total amount of releasable TXB_2 in both the presence and absence of CsA, 20 μmol/L calcium chloride was added to the initial aliquots, and the citrated blood was allowed to clot at 37°C for 30 minutes. Following centrifugation, the serum was separated and stored at −70°C until analyzed for the total amount of TXB_2 generated. The amount of TXB_2 released in both PRP and serum preparations was assessed by radioimmunoassay (R&D Systems, Abingdon, UK), measured by calculating the percentage of iodinated TXB_2 bound to a protein precipitate, and read from a standard curve. All assays were performed in duplicate.

1.6. Chemicals

Cyclosporin A (Sandimmun Neoral) was supplied by Novartis Farma, Lisbon, Portugal. ADP and collagen were obtained at Chrono-log Corp., Havertown, PA, USA. A thromboxane B_2 radioimmunoassay kit was purchased from R&D Systems, Abingdon, UK. All the other chemicals were of the highest analytical grade and were obtained from Sigma, St. Louis, MO, USA.

1.7. Statistical Analysis

Data are expressed as means±SEM of n experiments. Groups were tested for differences by using ANOVA and Student's t test.

2. Results

2.1. Blood Pressures

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) measurements were performed in all rats of the control and CsA-treated groups, before (0 week) and 2 and 7 weeks after initiating the CsA administrations. The DBP increased significantly after 2 and 7 weeks of treatment in the CsA-treated groups, especially in the CsA(5) group (week 2: 96±1, 119±2, and 114±1 mm Hg; week 7: 103±1, 114±2, and 112±1 mm Hg) ($p<0.05$) for control, CsA(5), and CsA(30), respectively (Table 1). Similar changes were recorded for the SBP. After 2 weeks, an increase in the SBP was registered in the CsA-treated groups (190±2 mm Hg for CsA5 and 179±1 mm Hg for CsA30), contrasting with the control (163±1 mm Hg; $p<0.05$). In week 7, the figures were approximately the same as those in week 2 (Table 1). The MBP also increased for the two CsA groups after 2 and 7 weeks, compared with the control (Table 1).

2.2. Platelet Serotonin Contents

The platelet serotonin measurements in the control and CsA-treated groups (5 and 30 mg/kg/day) were

Table 1. The effect of CsA on blood pressure after 2 and 7 weeks of treatment

Blood pressure (mm Hg)	Treatment (weeks)	Control (untreated)	Treated with CsA(5)	Treated with CsA(30)
SBP	0	165±1	165±3	161±1
	2	163±1	190±2*	179±1*
	7	164±1	189±3*	178±1*
DBP	0	100±2	89±5	100±2
	2	96±1	119±2*	114±1*
	7	103±1	114±2*	112±1*
MBP	0	121±2	112±4	120±1
	2	118±1	142±2*	136±1*
	7	120±3	136±4*	134±1*

Data are expressed as means±SEM of 20 separate values. SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure. CsA(5), cyclosporin A (5 mg/kg/day); CsA(30), cyclosporin A (30 mg/kg/day).
 *$p<0.05$ between the control group and each of CsA-treated group.

performed in week 0 (before starting the administrations) and in weeks 2 and 7. In week 0, the platelet 5-HT concentration of the control group was similar to that of the CsA-treated groups (Figure 1). In week 2, there was a decrease in the platelet serotonin contents of the CsA-treated groups in contrasting to the control group [in ng/mL: 1105±41 for control, 899±25 for CsA(5) and 698±21 for CsA(30); $p<0.05$]. Similar results were obtained in week 7: 999±24 for control, 877±17 for CsA(5), and 672±19 ng/mL for CsA(30), $p<0.05$ (Figure 1).
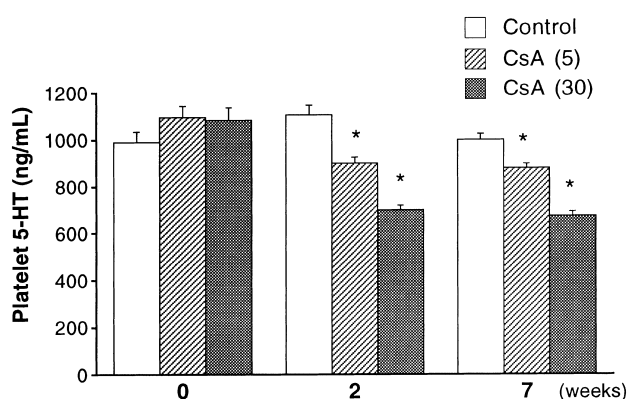

Fig. 1. The effect of CsA on rat platelet serotonin contents after 2 and 7 weeks of treatment. Data are expressed as means±SEM of 10 separate values: CsA(5), cyclosporin A (5 mg/kg/day); CsA(30), cyclosporin A (30 mg/kg/day). An asterisk indicates the difference ($p<0.05$) between the control group and each of CsA-treated group.

2.3. Whole Blood Platelet Aggregation

We tested the effects of cyclosporin A on whole blood platelet aggregation induced by collagen (5 μg/mL) and ADP (1.0 μmol/L), after 2 and 7 weeks of treatment. Before the start of CsA administrations, similar values were obtained for platelet aggregation in the control and the CsA-treated groups. Thus, only one mean is presented in week 0, which expresses values from the three groups (Table 2). After 2 weeks of treatment, no statistically significant increases in ADP-induced whole blood platelet aggregation were identified. In the CsA-treated groups, values for collagen-induced aggregation were significantly increased after the second week of treatment compared to those of the control group. These changes were most noticeable in the group that received 30 mg/kg/day of CsA. The results after 7 weeks of administration more or less confirmed those obtained after the second week (Table 2).

2.4. Platelet Thromboxane B_2 Generation

Baseline supernatant TXB_2 levels were 251.8±7.9 ng/mL in control platelets compared to 315.6±8.3 ng/mL and 304.5±8.1 ng/mL ($p<0.05$) in CsA-treated platelets, trough and peak, respectively (Figure 2). In ADP-stimulated platelets, TXB_2 release increased in the 0.1 and 1.0 μmol/L CsA-treated platelets compared to control. When platelets were stimulated with collagen, there was a sig-

Table 2. The effects of CsA on whole blood platelet aggregation and platelet count and volume after 2 and 7 weeks of treatment

Treatment (week)	Rat group	Aggregation (Ohms)		Platelet ($\times 10^9$/L)	MPV (fL)
		ADP (1.0 μmol/L)	Collagen (5 μg/mL)		
0		16.0±0.5	10.6±0.6	611±16	5.3±0.1
2	Control	16.1±0.5	10.1±0.4	631±31	5.4±0.2
	CsA(5)	17.1±1.1	13.4±1.0*	611±18	5.6±0.1
	CsA(30)	15.9±0.7	16.5±1.1*	609±27	5.6±0.2
7	Control	15.0±0.6	12.0±1.5	598±32	5.4±0.1
	CsA(5)	15.8±0.9	13.3±0.8*	573±24	5.5±0.2
	CsA(30)	15.4±0.5	17.3±0.9*	603±21	5.6±0.1

Data are expressed as means±SEM of 10 separate values. ADP, adenosine-5'-diphosphate; MPV, mean platelet volume; CsA(5), cyclosporin A (5 mg/kg/day); CsA(30), cyclosporin A (30 mg/kg/day).

*$p<0.05$ between the control group and each of CsA-treated group.

nificant increase in TXB$_2$ release for both CsA concentrations compared to control (Figure 2). Serum TXB$_2$ levels that were generated in control and CsA-treated whole blood allowed to clot were 321±10.4 ng/mL for control and 342±12.3 and 297±9.4 ng/mL ($p<0.05$) for 0.1 and 1.0 μmol/L of CsA (not shown).

2.5. Platelet Count and Mean Platelet Volume

As control parameters, we also counted the platelets and measured the mean platelet volume. No significant differences were obtained between the

Fig. 2. In vitro effect of CsA on rat platelet TXB$_2$ generation. Data are expressed as means±SEM of five separate values (performed in duplicate): CsA (0.1), cyclosporin A (0.1 μmol/L); CsA (1.0), cyclosporin A (1.0 μmol/L). An asterisk indicates the difference ($p<0.05$) between control platelets and platelets treated with each of CsA concentration.

three groups of rats at any stage of the study (Table 2).

3. Discussion

The present investigation was aimed at studying the peripheral serotonergic system, platelet aggregation, and blood pressure of rats treated with cyclosporin A at 5 and 30 mg/kg/day after 2 and 7 weeks of administration. These CsA concentrations were chosen because they corresponded to different stages of the CsA absorption in clinical practice. Therefore, 30 mg/kg/day of CsA administered to a rat represents the peak value of CsA achieved in human blood immediately following an intake of a daily dose, whereas 5 mg/kg/day in the rat corresponds to the trough concentration after blood concentration stabilization. The choice of the 2- and 7-week time periods was based on the fact that 2 weeks could correspond to an acute situation and 7 weeks to a chronic situation. The blood pressures rose significantly in the rats of the CsA-treated groups (5 and 30 mg/kg/day) after both 2 and 7 weeks of administration. So, as we initially expected, both trough and peak concentrations were really effective in increasing blood pressures.

Serotonin, due to its proaggregatory and also vasoconstrictor properties, may play an important role in the thromboembolic and hypertensive effects of CsA. Platelets are the main storage site of 5-HT and changes in their contents may reflect important physiological alterations, especially re-
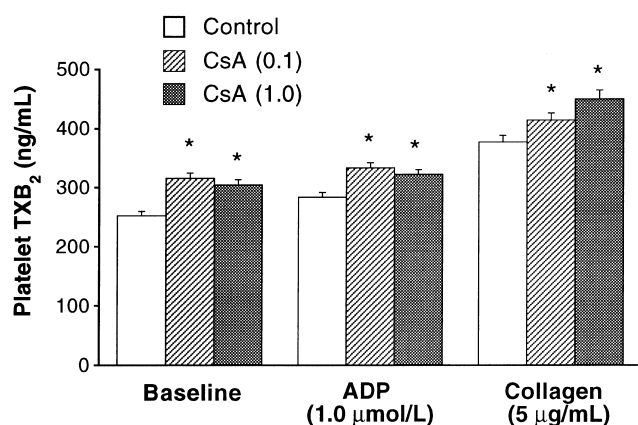
garding thrombotic and hypertensive effects. In this study we have identified a decrease in platelet 5-HT contents in both CsA-treated groups. Similar results were already obtained by others in a CsA treatment patient situation [14] and also in other hypertensive situations [25–27]. Low platelet serotonin concentration may be due to decreased uptake or increased release, as was documented in other studies, both in CsA [14] and non-CsA induced hypertension [25,28]. If serotonin is abnormally released by platelets, an increase in the plasma concentration of this amine could be expected and consequently an increased deposition on collagen fibres of the vessel wall, thus promoting direct vasoconstriction and platelet aggregation amplification. In agreement with our hypothesis, several papers have already reported that the increase in whole blood and plasma 5-HT is intensified during CsA treatment [14,20,22] and in other hypertensive situations as well [25,27]. On the other hand, cyclosporin A induces endothelial damage [29,30] that may contribute to increased vasoconstrictor response and platelet aggregation [31].

Thus, considering the above-mentioned information, an increase in platelet aggregation with CsA treatment is expected. In our study, whole blood platelet aggregation induced by collagen, but not by ADP, was increased in the rats of the CsA-treated groups when compared with control, as also was shown in other studies carried out by other investigators. In kidney transplant recipients, Malyszko et al. [14] found that CsA increased collagen- and ADP-induced whole blood and PRP aggregation when compared with healthy volunteers. However, by ADP stimulation whole blood and PRP aggregations were not statistically significant, as was also confirmed by Taylor et al. [32]. Mysliwiec et al. [21] indicated a rise in ADP-induced and 5-HT-augmented platelet aggregation in uremic rats, injected subcutaneously with 5 mg/kg/day of CsA after 2 and 4 weeks of administration, but did not find statistical significance in nonuremic rats treated with CsA when compared with the control. Grace et al. [12] demonstrated that platelet aggregation was increased in response to subthreshold doses of several agonists, such as ADP and epinephrine, in in vitro studies with cyclosporin A concentrations reflecting levels achieved in clinical practice [33,34]. They also found that the intake of a single dose of CsA in normal volunteers caused

maximal platelet activation after 2 hours but reverted to normal after 4 hours, thus suggesting that sustained exposure is needed to activate platelets consistently. In fact, they did not observe the same reversion in patients taking cyclosporin A: platelet activation at trough levels increased, but underwent a further increase at peak concentrations. Their results are in agreement with those obtained by Vanrenterghem et al. [8], who point out a positive link between CsA levels and enhanced aggregation. Our results confirmed the nonsignificant increases in ADP-induced aggregation and the significant increases in aggregatory response to collagen, already obtained by Malyszko et al. [14] and Taylor et al. [32], and the positive link between CsA levels (trough and peak) and increases in collagen-induced aggregation, extending the results obtained by Grace et al. [12] and Vanrenterghem et al. [8].

Confirming a platelet state of hyperactivity, we have obtained significant increases in platelet TXA_2 release at baseline and by platelet stimulation with both ADP and collagen. Peak-trough differences were observed by collagen stimulation in agreement with aggregation results and also with platelet 5-HT content results, thus suggesting a proportional relationship between CsA levels, thromboxane, and 5-HT release and platelet aggregation.

In conclusion, this study points out that both CsA concentrations that we tested influence blood pressure, platelet serotonin, and thromboxane contents and aggregation. Increased platelet aggregation, together with increased proaggregating and vasoconstricting agents concentration (at least 5-HT and TXA_2), near a damaged and/or dysfunctional vessel wall might provide the conditions to intensify vasoconstriction and platelet thrombus formation, which may certainly contribute to the development and/or maintenance of hypertension and increased risk of thromboembolic complications.

References

1. Borel JF, Di Padova F, Mason J, Quesniaux V, Ryffel B, Wenger R. Pharmacology of cyclo-

sporine (Sandimmune). I. Introduction. Pharmacol Rev 1989;41:239–42.

2. Kahan BD. Cyclosporine. N Engl J Med 1989;321:1725–38.

3. Langford CA, Klippel JH, Balow JE, James SP, Sneller MC. Use of cytotoxic agents and cyclosporine in the treatment of autoimmune diseases. Ann Intern Med 1998;129:49–58.

4. Schachter M. Cyclosporine A and hypertension. J Hypertens 1988;6:511–6.

5. Mimran A, Mourad G, Ribstein J, Halimi J-H Cyclosporin-associated hypertension. In: Laragh JH, Brenner BM, editors. Hypertension: Pathophysiology, Diagnosis and Management. New York: Raven Press; 1995. p. 2459–69.

6. Sander M, Victor RG. Hypertension after cardiac transplantation: pathophysiology and management. Curr Opin Nephrol and Hypertens 1995;4:443–51.

7. Ventura HO, Malik FS, Mehra MR, Stapleton DD, Smart FW. Mechanisms of hypertension in cardiac transplantation and the role of cyclosporine. Curr Opin Cardiol 1997;12:375–81.

8. Vanrenterghem Y, Roels L, Lerut T, Gruwez L, Michielsen P, Gresele P, Deckmyn H, Colucci M, Arnout J, Vermylen J. Thromboembolic complications and haemostatic changes in cyclosporin-treated cadaveric kidney allograft recipients. Lancet 1985;I:999–1002.

9. Muraki T, Sasaki Y, Gidding JC, Ishii H, Kaneko T, Yamamoto J. Antithrombotic effects of FK506 versus prothrombotic effect of cyclosporine in vivo. Transplantation 1995;60:308–9.

10. Miller BW, Hmiel SP, Schnitzler MA, Brennan DC. Cyclosporine as cause of thrombotic microangiopathy after renal transplantation. Am J Kidney Dis 1997;29:813–4.

11. Oskarsson HJ, Hofmeyer TG, Olivari MT. Cyclosporine impairs the ability of human platelets to mediate vasodilation. Hypertension 1997;29:1314–21.

12. Grace AA, Barradas MA, Mikhailidis DP, Jeremy JY, Moorhead JF, Sweny, P, Dandona P. Cyclosporine A enhances platelet aggregation. Kidney Int 1987;32:889–95.

13. González-Correa JA, De La Cruz JP, Lucena MI, Sánchez de la Cuesta F. The effect of cyclosporine A on platelet aggregation and thromboxane/prostacyclin balance in a model of ex-

trahepatic cholestasis in the rat. Thromb Res 1996;81:367–81.

14. Malyszko J, Pawlak D, Pawlak K, Malyszko JS, Buczko W, Azzadin A, Mysliwiec M. Platelet aggregation and peripheral serotonergic system in kidney transplant recipients treated with cyclosporine. Transplant Proc 1996;28:1954–7.

15. Jorkasky DK, Fisher CA, Stahl RF, Addonizio VP, Glickman JD. The effects of cyclosporine on human platelet aggregation and thromboxane release. Transplant Proc 1989;21:948–9.

16. Bossaller C, Forstermann U, Hertel R, Olbsricht C, Reschke V, Fleck E. Cyclosporin A inhibits endothelium-dependent vasodilatation and vascular prostacyclin production. Eur J Pharmacol 1989;165:165–9.

17. Baker LRI, Tucker B, Kovacs IB. Enhanced in vitro hemostasis and reduced thrombolysis in cyclosporine-treated renal transplant recipients. Transplantation 1990;49:905–9.

18. Levi M, Wilmink J, Buller HR, Surachno J, Ten Cate JW. Impaired fibrinolysis in cyclosporin-treated renal transplant recipients. Transplantation 1992;54:978–83.

19. Cohen H, Neild GH, Patel R, Mackie IJ, Machin SJ. Evidence for chronic platelet hyperaggregability and in vivo activation in cyclosporin-treated renal allograft recipients. Thromb Res 1988;49:91–101.

20. Ueda D, Suzuki K, Malyszko J, Pietraszek MH, Takada Y, Takada A, Kawabe K. Fibrinolysis and serotonin under cyclosporine A treatment in renal transplant recipients. Thromb Res 1994;76:97–102.

21. Mysliwiec J, Azzadin A, Chabielska E, Takada A, Mysliwiec M, Buczko W. The effect of tacrolimus (FK506) and cyclosporin A (CyA) on peripheral serotonergic mechanisms in uremic rats. Thromb Res 1996;83:175–81.

22. Ueda D, Suzuki K, Malyszko J, Pietraszek MH, Takada Y, Takada A, Kawabe K. Serotonergic measures in cyclosporine A treated rats. Thromb Res 1994;76:171–9.

23. Vanhoutte PM. Serotonin, hypertension and vascular diseases. Neth J Med 1991;38:35–42.

24. Azzadin A, Mysliwiec J, Wollny T, Mysliwiec M, Buczko W. Serotonin is involved in the pathogenesis of hypertension developing during erythropoietin treatment in uremic rats. Thromb Res 1995;77:217–24.

25. Hervé P, Launay JM, Scrobohaci ML, Brenot F, Simonneau G, Petitpretz P, Poubeau P, Cerrina J, Duroux P, Drouet L. Increased plasma serotonin in primary pulmonary hypertension. Am J Med 1995;99:249–54.

26. Ding YA, Chou TC, Huan R. Are platelet cytosolic calcium, serotonin concentration and blood viscosity different between hypertensive and normotensive subjects? Cardiology 1994; 85:76–81.

27. Nityanand S, Tekwani BL, Chandra M, Shanker K, Singh BN. Kinetics of serotonin in platelets in essential hypertension. Life Sci 1990;46:367–72.

28. Jafri SM, Chandra M, Dhawan S, Sonid D, Chandra M, Shanker K. Factors influencing platelet serotonin uptake in essential hypertension. Int J Cardiol 1992;34:327–33.

29. Evans SM, Giddings JC, Muraki T, Yamamoto J. Expression of von Willebrand factor, P-selectin (CD62P) and thrombomodulin in human endothelial cells in culture modulated by cyclosporin A. Clin Lab Haematol 1997;19: 115–22.

30. Bombeli T, Muller M, Straub PW, Haeberli A. Cyclosporine-induced detachment of vascular endothelial cells initiates the intrinsic coagulation system in plasma and whole blood. J Lab Clin Med 1996;127:621–34.

31. Vanhoutte PM. Platelets, endothelium and blood vessel wall. Experientia 1988;44:105–8.

32. Taylor JE, Scott N, Hill A, Bridges A, Henderson IS, Stewart WK, Belch J. Oxygen free radicals and platelet and granulocyte aggregability in renal transplant patients. Transplantation 1993;55:500–4.

33. Niedelberger W, Schaub BP, Beveridge T. High-performance liquid chromatographic determination of cyclosporin A in human plasma and urine. J Chromatogr 1980;182:454–8.

34. Reynolds DJM, Aronson JK. Cyclosporin. Br Med J 1992;305:1491–4.