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In-vivo Distribution and Erythrocyte Binding Characteristics of Cyclosporin in Renal Transplant Patients

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Abstract

The pharmacokinetic parameters of cyclosporin, a potent immunosuppressive agent, show large intra- and inter-individual variability, possibly because of the different analytical methods used. A recently developed cyclosporin-specific radioimmunoassay has been used to study the in-vivo distribution and binding characteristics of cyclosporin in whole blood, plasma and erythrocytes of fifteen renal transplant patients.

The profiles of cyclosporin concentration–time curves after an oral dose of cyclosporin had either one peak (ten patients, group A) or two (five patients, group B). Essentially no difference was observed between the two groups in the relationship between equilibrium cyclosporin concentrations in erythrocyte and plasma as a function of whole-blood concentration. The equilibrium in-vivo cyclosporin concentrations in erythrocytes and plasma were, however, markedly lower than those previously observed under in-vitro conditions. The ratio of cyclosporin concentration in erythrocytes (C_E) to that in plasma (C_P) changed with time, in inverse proportion to the change in cyclosporin concentration in blood, over the range 0·63–2·80 in individual patients with an average of $1·36 \pm 0·07$ (mean ± s.e.m.) for group A and $1·42 \pm 0·23$ for group B. The apparent cyclosporin binding affinity (K_d) to erythrocytes under in-vivo conditions averaged $452·2 \pm 47·6$ nM ($543·5 \pm 57·2$ ng mL^{-1}) for group A and $419·4 \pm 41·2$ nM ($504·1 \pm 49·5$ ng mL^{-1}) for group B, whereas apparent cyclosporin binding capacity (B_{max}) of the blood cell averaged $0·83 \pm 0·07$ nmol mL^{-1} for group A and $0·78 \pm 0·07$ nmol mL^{-1} for group B. Significantly reduced average K_d ($262·7 \pm 40·2$ nM or $315·8 \pm 48·9$ ng mL^{-1}, $P < 0·01$) and B_{max} ($0·56 \pm 0·08$ nmol mL^{-1}, $P < 0·05$) values were observed during the period after T_{max} (4–12 h after the drug ingestion) in group A patients. Apparent K_d and B_{max}, determined by a nonlinear regression technique, were $131·6 \pm 29·4$ and $1088·0 \pm 114·7$ nM ($158·2 \pm 35·4$ and $1307·8 \pm 137·9$ ng mL^{-1}) and $0·178 \pm 0·024$ and $0·814 \pm 0·078$ nmol mL^{-1}, respectively, during the 4–12 h period in group A patients.

These findings reveal distinct differences in in-vivo distribution of cyclosporin and the binding characteristics of the compound to erythrocytes from those previously observed under in-vitro conditions. The significantly lower K_d of cyclosporin binding to erythrocytes during the elimination phase suggests a potential effect of cyclosporin-containing erythrocytes or of cyclosporin contained in erythrocytes during cyclosporin treatment.

Distribution of cyclosporin, a potent immunosuppressive agent, in human blood components has been studied extensively (Lemaire & Tillement 1982; Atkinson et al 1983; Mraz et al 1983; Niederberger et al 1983; Gurecki et al 1985; Rosano 1985; Annesley et al 1986; Sgoutas et al 1986; Legg & Rowland 1987; Hamberger et al 1988; Legg & Rowland 1988a, b; Urien et al 1990; Hughes et al 1991). The presence of a cyclophilin A-like cyclosporin binding protein in erythrocytes has been confirmed (Foxwell et al 1988) and plasma lipoproteins were found to bind significant amounts of cyclosporin (Lemaire & Tillement 1982; Mraz et al 1983; Gurecki et al 1985; Sgoutas et al 1986; Urien et al 1990; Hughes et al 1991). Reversible and saturable uptake of cyclosporin by peripheral blood lymphocytes has also been reported (Ryffel et al 1982; LeGrue et al 1983). These characteristic profiles of cyclosporin binding to various blood components may influence the pharmacokinetic behaviour of cyclosporin and thus the estimate of drug bioavailability. The pharmacokinetic parameters of cyclosporin are, in fact, known to show large intra- and inter-individual variability. Fahr (1993) recently claimed that an important factor in the variability of cyclosporin pharmacokinetics is the different analytical methods used; the same author also pointed out that measurement of cyclosporin in different biological fluids (blood, plasma, serum) added further variability to the pharmacokinetic data previously published. Previous studies on distribution and binding of cyclosporin in human blood components have, on the other hand, been performed under in-vitro or ex-vivo conditions and the distribution of the drug to erythrocytes or non-plasma components was often estimated indirectly in combination with calculation (Lemaire & Tillement 1982; Niederberger et al 1983; Agarwal et al 1985; Lemaire et al 1986; Legg & Rowland 1988 a, b; Legg et al 1988; Hamberger et al 1988; Akagi et al 1991).

The recent development of a cyclosporin-specific radioimmunoassay using a monoclonal antibody has provided a valid and efficient tool for quantitative analysis of cyclosporin in blood. Taking advantage of this methodology we have attempted, by direct measurement of the drug in the whole blood, plasma and erythrocytes of renal transplant patients, to analyse the characteristics of in-vivo distribution of cyclosporin and the binding of the compound to erythrocytes.

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Materials and Methods

Subjects

Fifteen renal transplant patients were given a single oral dose of cyclosporin solution (5·0 mg/50 mL; Sandoz Ltd) at 0800 h

Table 1. Characteristics of fifteen male renal transplant patients.

Patient	Age	Cyclosporin dose (mg kg^{-1}/12 h)	Days after transplantation	Haematocrit (%)	Creatinine clearance (mL min^{-1})
Group A					
1	12	5·40	9	26·2	90·4
2	56	2·90	9	25·7	56·0
3	33	3·34	9	29·0	50·6
4	31	2·17	9	23·3	52·0
5	29	2·66	12	30·1	30·8
6	46	3·85	13	28·5	52·3
7	50	1·45	31	33·1	81·5
8	36	2·38	36	30·8	48·4
9	29	2·09	40	24·9	29·4
10	31	2·23	44	29·3	54·7
Average 35·3 ± 4·0 (mean ± s.e.m.)				28·1 ± 1·0	54·6 ± 6·0*
Group B					
11	35	3·06	8	27·8	64·2
12	50	2·90	8	36·08	0·7
13	44	2·92	9	23·68	0·7
14	36	2·63	16	27·3	110·8
15	44	3·00	36	32·0	90·5
Average 41·8 ± 2·8 (mean ± s.e.m.)				29·3 ± 2·1	85·4 ± 7·6*

*$P < 0.001$.

after the hospital breakfast. The dose given to each patient is shown in Table 1 with other patient data. Informed consent was obtained from all the patients.

Blood sample preparation

Blood samples (10 mL) were collected in preheated (37°C) tubes containing EDTA (final concentration 0·1%) 0, 1, 2, 3, 4, 5, 7, 9 (for some patients) and 12 h after cyclosporin ingestion. One third of each blood sample was frozen and the rest was immediately centrifuged at 35–37°C at 1600 *g* for 5 min to separate the plasma. The buffy coat was removed from the remaining erythrocytes. Plasma and erythrocytes were stored frozen. The haematocrit was measured using a Total Haematology Management System H2 (Bayer-Sankyo Ltd).

Radioimmunoassay

Cyclosporin concentrations in blood samples were measured by means of a cyclosporin-specific radioimmunoassay. Each of the thawed whole-blood, erythrocyte and plasma samples (200 μL) was extracted with methanol (800 μL). After centrifugation, the supernatant (50 μL) was submitted to a double-antibody radioimmunoassay by Cyclo-Trac RIA kit with mouse monoclonal anti-cyclosporin antibody (Lot No. 9215030, Incstar Corp.) and [^{125}I]histaminylsuccinyl cyclosporin C (Lot No. 9222014, Incstar Corp.) as labelled antigen. The minimum detectable dose of cyclosporin was 4·0 ng mL^{-1}. The cross-reactivities of cyclosporin metabolites M-1, M-17 and M-21 had been proved to be 3, < 1 and 5%, respectively. Intra- and inter-assay coefficients of variation were within 7%. Analytical recoveries of cyclosporin added to blood samples varied between 89·5 and 108·2% depending on the doses used.

Pharmacokinetic parameters

The parameters measured were the area under the cyclosporin concentration–time curve between times 0 and 12 h (ng h mL^{-1}; denoted AUC) and the highest concentration observed in eight samples taken from a patient during the 12-h period after cyclosporin injection (ng mL^{-1}; denoted C_{max}). The cyclosporin concentration at 0 h is referred to as the trough level (ng mL^{-1}). Cyclosporin concentrations in erythrocytes and plasma contained in 1 mL whole blood were also obtained by converting the measured cyclosporin concentrations in the respective blood components on the basis of the haematocrit values. These concentrations were designated C_E and C_P, respectively.

Scatchard analysis

Scatchard analysis (Meyts & Roth 1975) was performed using C_E and C_P. Regression lines were obtained by the method of least squares.

Statistics

Statistical analyses were performed done using Student's paired *t*-test and significance was defined as $P < 0.05$.

Results

The combined values of the measured cyclosporin concentrations in plasma and erythrocytes corresponded, after correction for haematocrit values, to 93·6 ± 0·6% (mean ± s.e.m., n = 118) of the cyclosporin concentration measured in whole blood. The cyclosporin concentration–time curves obtained could be divided into two groups, one showing a single early peak (T_{max} 1–3 h; 10 patients, group A) the other a double peak (T_{max} 1 and 4–5 h) or a single late peak (T_{max} 4–5 h) (five patients, group B). Fig. 1 indicates the time courses for the average cyclosporin concentrations in whole blood, plasma and erythrocytes of patients from groups A and B. In each group, the time course profiles from the different samples (whole blood, plasma and erythrocytes) were essentially parallel.

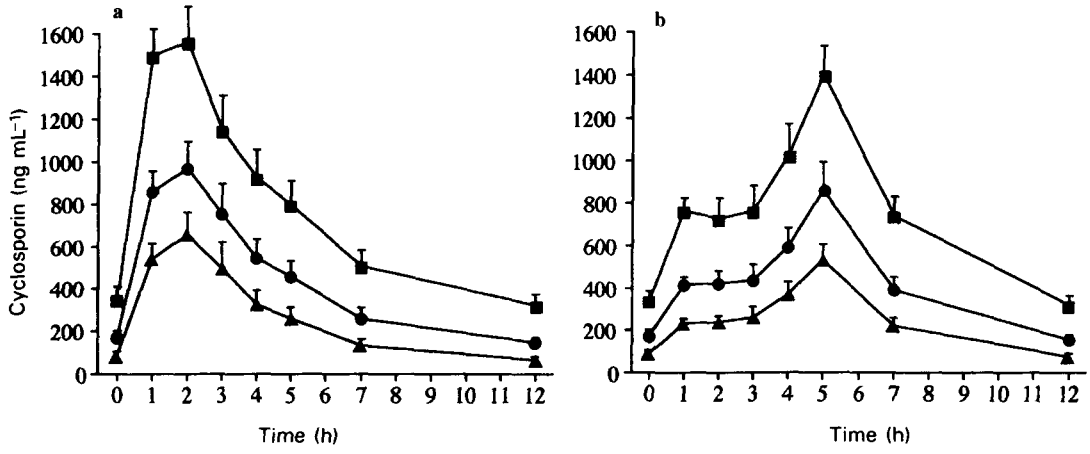

FIG. 1. Cyclosporin concentration–time courses after oral dosing in whole blood (●), erythrocytes (■), and plasma (▲), of renal transplant patients of group A (a) and group B (b). Results are mean ± s.e.m., n = 10 (a) and 5 (b).

Patients' characteristics are listed in Table 1; except for the average values of creatinine clearance distinct differences were not observed between groups A and B.

Table 2 shows the average pharmacokinetic parameters of cyclosporin in the patients examined. Significant differences between the results from groups A and B were observed only in T_{max}. Fig. 2 illustrates the relationship between equilibrium cyclosporin concentrations in erythrocytes and plasma as a function of whole blood concentration in patients from groups A and B. From Fig. 2, the cyclosporin concentration level at which erythrocytes in blood were saturated was estimated to be lower than 1 nmol mL^{-1} in both groups of patients.

The ratio of cyclosporin concentration in erythrocytes (C_E) to that in plasma (C_P) changed with time within the range 0·63–2·80 in individual patients; the average ratios were 1·36 ± 0·07 (mean ± s.e.m., n = 78) for group A and 1·42 ± 0·23 (n = 40) for group B, with no statistically significant difference. The time change profiles of C_E/C_P are shown in Fig. 3. The C_E/C_P values at 0 h, 1·66 ± 0·09 (n = 10) for group A and 1·90 ± 0·19 (n = 5) for group B, decreased to minimal levels (1·01 ± 0·07 (n = 10) for group A and 1·18 ± 0·20 (n = 5) for group B) at the time corresponding to T_{max} in both groups and then increased to 1·79 ± 0·28 (n = 10) for group A and 1·87 ± 0·28 (n = 5) for group B, 12 h after dosing. In group B, an additional trough of C_E/C_P, 1·32 ± 0·22 (n = 5), was observed at the time of the first T_{max}. The values of C_E/C_P at a time 1 ~ 5 h after cyclosporin ingestion in group A (Fig. 3a) and 1 ~ 7 h after cyclosporin ingestion in group B (Fig. 3b) were significantly lower than those at 0 h.

Cyclosporin in-vivo binding characteristics of erythrocytes in the patients (Table 3) were analysed by Scatchard plot using the directly measured values of C_E and C_P. In this analysis, cyclosporin distribution among blood components other than erythrocytes and plasma and cyclosporin binding to plasma proteins were not taken into consideration (Lemaire & Tillement 1982; Lemaire et al 1986; Legg & Rowland 1988b; Legg et al 1988). Typical C_E/C_P vs C_E curves for patients in groups A and B are shown in Fig. 4. For each patient in group A, C_E/C_P vs C_E points obtained during the period before T_{max} (increasing phase of cyclosporin concentration) did not fit the regression curves constructed with the points obtained during the period after T_{max} (decreasing phase of cyclosporin concentration). Apparent cyclosporin binding affinity (K_d) of erythrocytes ranged from 270·4 to 665·8 nM (from 325·0 to 800·3 ng mL^{-1}) with an average of 452·2 ± 47·6 nM (543·5 ± 57·2 ng mL^{-1}) (mean ± s.e.m., n = 10) for group A

Table 2. Cyclosporin pharmacokinetic parameters in whole blood, erythrocytes and plasma of renal transplant patients.

	Blood sample	Group A	Group B
Trough level (ng mL^{-1})	Whole blood	170·5 ± 31·1	167·6 ± 29·4
	Erythrocytes	353·0 ± 58·8	337·6 ± 45·2
	Plasma	86·8 ± 16·9	84·8 ± 16·2
T_{max} (h)	Whole blood	1·6 ± 0·2	4·6 ± 0·2*
	Erythrocytes	1·5 ± 0·2	4·6 ± 0·2*
	Plasma	1·9 ± 0·2	4·6 ± 0·2*
C_{max} (ng mL^{-1})	Whole blood	1073·7 ± 129·3	874·2 ± 127·0
	Erythrocytes	1724·6 ± 148·3	1420·5 ± 131·4
	Plasma	735·3 ± 117·4	559·0 ± 62·9
AUC (ng h^{-1} mL^{-1})	Whole blood	5187·2 ± 674·6	4929·8 ± 452·0
	Erythrocytes	9061·8 ± 1038·2	8828·9 ± 574·6
	Plasma	2980·8 ± 460·5	2787·2 ± 271·8

Mean ± s.e.m. (n = 5–10). *$P < 0.001$ compared with the corresponding values for group A.

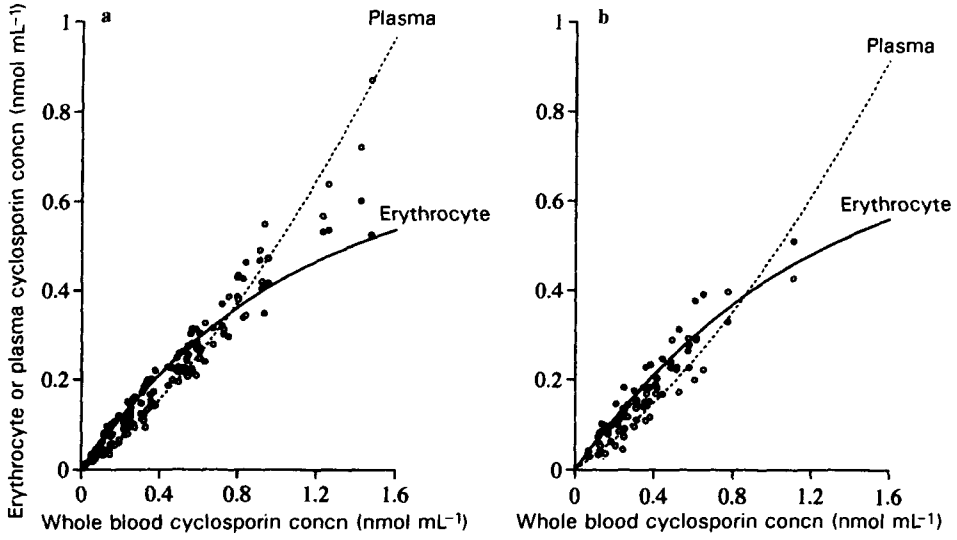

FIG. 2. Relationship between equilibrium cyclosporin concentrations in erythrocytes (●) and plasma (○) as a function of whole blood concentration in group A (a) and group B (b).

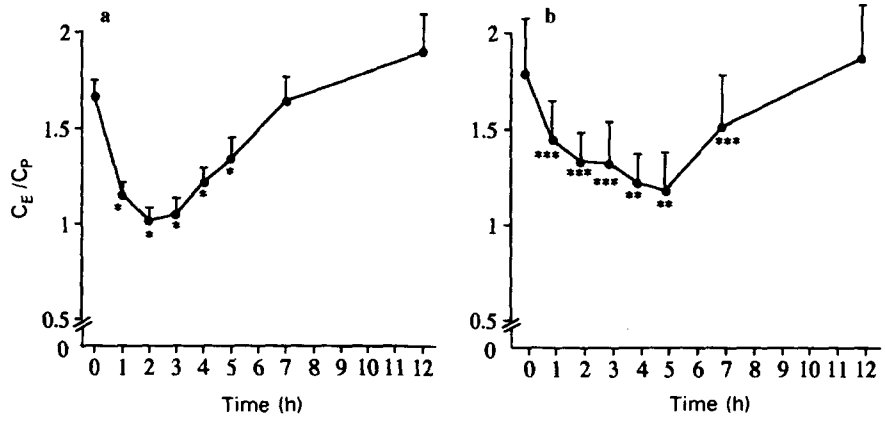

FIG. 3. Time courses of the ratio of cyclosporin concentrations in erythrocytes (C_E) and plasma (C_P) after oral dosing in renal transplant patients of group A (a) and group B (b). Results are mean ± s.e.m., n = 10 (a) and 5 (b). $^*P < 0.001$, $^{**}P < 0.01$, $^{***}P < 0.05$ compared with values at 0 h.

patients and from 266·2 to 496·4 nM (from 320·0 to 596·7 ng mL^{-1}) with an average of 419·4 ± 41·2 nM (504·1 ± 49·5 ng mL^{-1}) (n = 5) for group B patients. The apparent cyclosporin binding capacity (B_{max}) of erythrocytes, on the other hand, varied from 0·56 to 1·31 nmol mL^{-1} (average 0·83 ± 0·07 nmol mL^{-1}, mean ± s.e.m., n = 10) for group A patients and from 0·63 to 1·01 nmol mL^{-1} (average 0·78 ± 0·07 nmol mL^{-1}, n = 5) for group B patients. The average values of the cyclosporin binding parameters obtained for the overall period (0–12 h after ingestion of the drug) did not differ significantly between groups A and B. In group A, however, both apparent K_d and B_{max} separately calculated with C_E and C_P observed during the period after T_{max} (4–12 h after cyclosporin ingestion) were 262·7 ± 40·2 nM (315·8 ± 48·3 ng mL^{-1}, n = 10) and 0·56 ± 0·08 nmol mL^{-1} (n = 10), respectively, which were both significantly lower ($P < 0.01$ and $P < 0.05$, respectively) than the average values given above for the overall period (0–12 h after cyclosporin ingestion). When a nonlinear regression technique was applied, apparent K_d and B_{max} of erythrocytes during the 4–12 h period were 131·6 ± 29·4 and 1088·0 ± 114·7 nM (158·2 ± 35·4 and 1307·8 ± 137·9 ng mL^{-1}) and 0·178 ± 0·024 and 0·814 ± 0·078 nmol mL^{-1}, respectively, suggesting the presence of two kinds of cyclosporin binding site of high and low affinity.

Discussion

Increasing data have been accumulated for the pharmacokinetics of cyclosporin in man (for a review see Fahr 1993). In this study we studied in-vivo cyclosporin–erythrocyte distribution and binding characteristics in renal transplant patients by directly measuring cyclosporin concentrations in erythrocytes and plasma as well as in whole blood, using a highly specific cyclosporin radioimmunoassay. The results show that the combined measured values of cyclosporin concentrations in erythrocytes and plasma equal 94% of the cyclosporin concentrations separately measured in whole blood, indicating

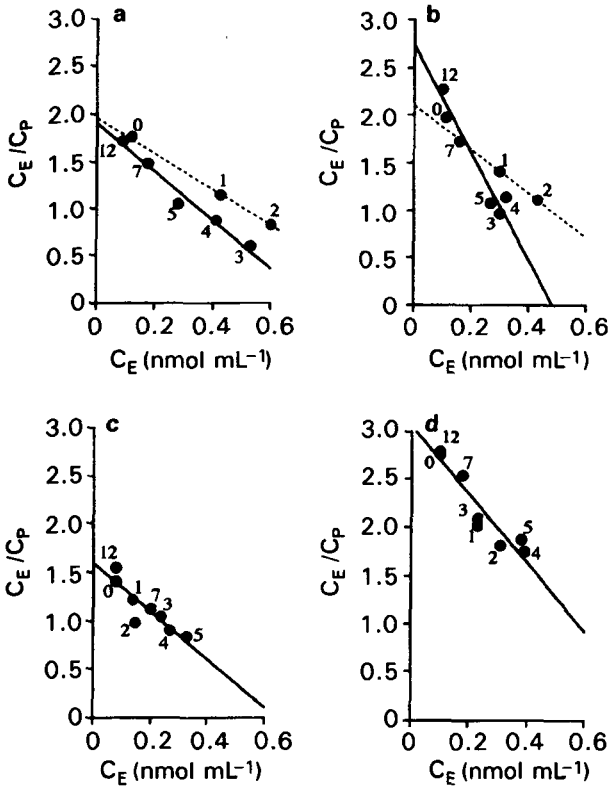

FIG. 4. Typical Scatchard plots of in-vivo cyclosporin binding to erythrocytes in two patients from group A (a and b) and two patients from group B (c and d). Numbers indicate hours after oral cyclosporin.

the distribution of about 6% of blood cyclosporin in leucocytes and other cells. These data are similar to those obtained in previous in-vitro experiments (Lemaire & Tillement 1982; Atkinson et al 1983; Niederberger et al 1983).

Double-peaks in the cyclosporin concentration–time curve have frequently been observed (Kahan et al 1983; Clardy et al 1988; Lindholm et al 1988; Phillips et al 1988; Reymond et al 1988). Phillips et al (1988) and Clardy et al (1988) described three distinct absorption patterns of cyclosporin: biphasic, slow and rapid. In this work the profiles observed in patients in groups A and B (Fig.1a, b) seem to correspond to those showing rapid and biphasic absorption patterns, respectively, although the single peak of cyclosporin concentration in group A appeared earlier (2–3 h after dosing) than that (4 h) in the patients examined by Phillips et al (1988). Henricsson et al (1989) have previously identified a sulphate conjugate of cyclosporin in human bile and plasma, suggesting that cyclosporin undergoes enterohepatic circulation. The idea of enterohepatic recycling of cyclosporin as a factor affecting cyclosporin absorption is, however, still controversial (Maurer & Lemaire 1986; Reymond et al 1988), and it has not yet been established as a cause of the second peak of cyclosporin concentration shown in the double-peak profile. The role of food in the appearance of the second peak of cyclosporin concentration has also been discussed (Lindholm et al 1988; Reymond et al 1988; Gupta et al 1990), but again this has not yet been fully proved. Phillips et al (1988) have found that adolescents who continued to maintain good renal graft function all fell into the biphasic profile group. The characteristics of patients examined in the current study (Table 1) were not significantly different between groups A and B, with the exception of creatinine clearance values. The implication of the significant difference observed in average creatinine clearance values in the two kinds of cyclosporin concentration–time curve profiles remains to be clarified.

The changes of cyclosporin concentrations with time in whole blood, erythrocytes and plasma were nearly parallel, and the highest concentration was consistently observed in erythrocytes (Fig. 1a, b). Experiments in-vitro have shown that the uptake of cyclosporin by erythrocytes is saturated above a cyclosporin concentration of 3000–5000 μg L^{-1} (Atkinson et al 1983; Niederberger et al 1983; Foxwell et al 1988). In this work the cyclosporin concentrations measured in blood samples were far lower than those hitherto reported. The relationship between in-vivo equilibrium concentrations of cyclosporin in erythrocytes and plasma as a function of whole blood (Fig. 2) was analogous to that observed in an in-vitro experiment by Niederberger et al (1983). This previous study, however, found that blood cells become saturated at blood concentrations above 5 μg mL^{-1}, whereas our results showed that the cyclosporin saturation levels in erythrocytes were lower than 1 nmol mL^{-1} ($\simeq$1 μg mL^{-1}) within the therapeutic range of cyclosporin concentration; this was proved by the cyclosporin binding capacity (B_{max}) values estimated by Scatchard analysis. The cyclosporin fractions bound to erythrocytes and other blood cells were reported to be about 60% and 10%, respectively (Niederberger et al 1983). These results clearly indicate, therefore, that marked differences exist between cyclosporin binding behaviour in blood under in-vivo and in-vitro conditions.

The distribution of cyclosporin between human erythrocytes and plasma has been extensively studied under in-vitro conditions using indirectly estimated data of cyclosporin concentrations in erythrocytes. The distribution ratio under in-vitro conditions was reported to be essentially constant (1·5)

Table 3. Characteristics of in-vivo cyclosporin binding to erythrocytes in renal transplant patients.

Group	Apparent K_d (nM)			Apparent B_{max} (nmol mL^{-1} blood)		
	Period before T_{max}[a]	Period after T_{max}[a]	Overall period	Period before T_{max}[a]	Period after T_{max}[a]	Overall period
A	552·3 ± 53·8*	262·7 ± 40·2*	452·2 ± 47·6	0·99 ± 0·08†	0·56 ± 0·08†	0·83 ± 0·07
B	422·4 ± 48·6	356·5 ± 52·8	419·4 ± 41·2	0·81 ± 0·09	0·70 ± 0·09	0·78 ± 0·07

[a]T_{max} for Group B represents the second peak of the double-peak profile of the cyclosporin concentration–time curve. On calculation, the cyclosporin concentration values at T_{max} were included in those in "period before T_{max}". Mean ± s.e.m. (n = 13–53), *$P < 0·001$, †$P < 0·001$.

over the therapeutic concentration range (Lemaire & Tillement 1982) or to be 0·5 ~ 1 at 37°C when the cyclosporin concentrations were below 1000 $\mu g\ L^{-1}$ (Niederberger et al 1983; Annesley et al 1986). Hamberger et al (1988) have found in ex-vivo experiments that the ratio of cyclosporin concentrations in blood cells and plasma was as high as 10·7 ± 3·6 (mean ± s.d.) in kidney transplant recipients. In this study, the in-vivo distribution ratios, C_E/C_P, changed in the range 0·63 ~ 2·80 during the 12-h period after cyclosporin ingestion, again indicating a distinct difference between cyclosporin behaviour in human blood under in-vivo and in-vitro conditions. We have previously shown that cyclosporin and its metabolites behave as stable complexes with erythrocytic components during gel chromatography under neutral conditions, whereas the strength of their binding to plasma proteins is such that complex formation cannot be detected during chromatography (Kadobayashi et al 1995; Yamamoto et al 1995). The apparent K_d values observed in this in-vivo study were comparable with those obtained by Agarwal et al (1985) (177 ~ 971 ng mL^{-1}) and by Hamberger et al (1988) (790 ng mL^{-1}), although these investigators determined cyclosporin concentrations in erythrocytes indirectly by combined ex-vivo experiment and calculation. Cyclosporin binding affinity to erythrocytes under in-vitro conditions was estimated to be much higher, i.e. 5×10^{-8} M (Agarwal et al 1986) and $5{\cdot}6 \times 10^{-8}$ M (Legg & Rowland 1988a). These values are rather closer to the K_d (15·3 nM at 4°C) of a erythrocytic cyclophilin A which we have purified (Yamamoto et al 1995). On the basis of the apparent B_{max} of cyclosporin binding to erythrocytes in-vivo, the number of cyclosporin molecules bound to an erythrocyte cell was estimated to be $1{\cdot}6 \times 10^5$ in group A and $1{\cdot}4 \times 10^5$ in group B, whereas Foxwell et al (1988) have estimated from in-vitro data that $2\text{–}2{\cdot}5 \times 10^5$ molecules of cyclosporin were bound per cell at a concentration of 3 $\mu g\ mL^{-1}$ (saturated).

It is noteworthy that significantly lower apparent K_d and B_{max} were observed in blood taken after T_{max} (descending phase of cyclosporin concentration) from patients in group A. On the other hand, we obtained, by tentative calculation, higher average apparent K_d (617·4 ± 150·6 nM or 742·1 ± 181·0 ng mL^{-1}, mean ± s.e.m., n = 5) and B_{max} (1·02 ± 0·16 nmol mL^{-1}, mean ± s.e.m., n = 5) in group A patients during the period 1–3 h after cyclosporin ingestion, although these cyclosporin binding parameters during the absorption phase may not be persuasive enough because of the limited number of data available for calculation. The equilibration of cyclosporin between erythrocytes and plasma occurs rapidly within 10–20 min, although under in-vitro conditions (Lemaire & Tillement 1982; Vine & Bowers 1987; Fahr 1993), so it seems improbable that such higher values of apparent K_d and B_{max} are due to the period of the absorption phase which is not long enough for the equilibration. Low-affinity non-saturable binding of cyclosporin to the erythrocyte membrane has been described (Foxwell et al 1988). Scatchard analysis by a nonlinear regression technique revealed higher values of K_d (1088·0 ± 114·7 nM or 1307·8 ± 137·9 ng mL^{-1}) and B_{max} (0·814 ± 0·078 nmol mL^{-1}) with lower K_d (131·6 ± 29·4 nM or 158·2 ± 35·4 ng mL^{-1}) and B_{max} (0·178 ± 0·024 nmol mL^{-1}) in group A patients during the 4–12 h period. Cyclosporin binding to such low-affinity sites of erythrocytes might to a greater extent be reflected in the higher K_d and B_{max} observed in group A patients during the early hours after cyclosporin ingestion. The drug bound to such low-affinity sites of erythrocytes may be released more easily or rapidly during the elimination phase to be available in pharmacology and transformation of cyclosporin, leaving the rest at high-affinity binding sites in erythrocytes intact. Although a similar phenomenon may or may not have taken place in cyclosporin binding to erythrocytes in group B patients, the double-peak profile of cyclosporin concentration-time curves made such analysis difficult.

In summary, this paper describes the in-vivo pharmacokinetic behaviour and erythrocyte-binding characteristics of cyclosporin in renal transplant patients. The findings disclose distinct differences in erythrocyte distribution and binding parameters of the drug under in-vivo condition from those previously observed in-vitro. In addition, the significantly lower K_d observed after T_{max} in group A patients supports the existence of some population of stable cyclosporin persistently bound to erythrocytes, which suggests possible pharmacological effects of the cyclosporin-containing erythrocytes or cyclosporin bound to erythrocytes during cyclosporin treatment through, for example, phagocytosis of such erythrocytes by Kupffer's cells. This possibility remains to be explored.

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