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Cyclosporin: Pharmacokinetics and Detailed Studies of Plasma and Erythrocyte Binding During Intravenous and Oral Administration

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Summary. On the basis that unbound concentration better correlates with response than total plasma or blood concentration, the inter- and intra-subject variability in the distribution of cyclosporin within blood and to plasma components was studied in renal transplant patients. Pharmacokinetic aspects were also studied.

Blood samples were analysed from patients who received the drug both by a 72-h i.v. infusion and orally (7 $mg \cdot kg^{-1}$ twice daily). Steady-state was reached within 18 h of starting the i.v. infusion; the plasma data were best fitted by a biexponential equation with half-times of 0.13-1.02 h and 4.3-13.9 h, associated with the two phases. The mean plasma clearance was 700 ml/min. Concentrations during the infusions measured by RIA and HPLC were comparable. Oral profiles showed rapid and extensive absorption. The peak plasma concentrations were 1460-1880 $\mu g \cdot l^{-1}$ and occurred 2-4 h after dosing, with bioavailability estimates of 41-113%. Concentrations measured by RIA were higher than by HPLC.

Blood-to-plasma concentration ratio measurements of cyclosporin at 37 °C decreased with increasing plasma concentration and increased with haematocrit. Fraction unbound, measured by ultracentrifugation, was in the range 0.042-0.122 with an average of 0.068, and varied little in some patients but showed systematic changes with time in others. Cyclosporin binding was found to be related not only to the triglyceride but, more particularly, to the cholesterol-related lipoproteins in plasma. Monitoring cholesterol may be helpful in identifying patients with extremes in binding or with widely varying binding.

Key words: cyclosporin; pharmacokinetics, infusions, binding, lipids, bioavailability

Cyclosporin (CyA) is a third generation immunosupressive agent which has been used successfully in organ transplantation in man since 1981. The drug is highly effective when dosed orally and in renal transplant patients an 80% 1-year graft survival has been demonstrated in several centres [1]. The drug is, however, not without side-effects, principally renal and hepatic toxicity which are thought to correlate with drug concentration in plasma or blood by some authors [2-4] while others are less convinced [5-9].

Accordingly, as the pharmacokinetics of CyA varies among patients, drug level monitoring is being increasingly used as a guide to therapy. Nevertheless, uncertainties exist as to what and where to measure, with most laboratories reporting concentrations in whole blood measured by radioimmunoassay (RIA). This choice of whole blood is because of the ease of analysis and because CyA and many of its metabolites (some of which cross-react in the RIA) concentrate preferentially into erythrocytes, in a temperature dependent manner, making plasma concentration measurements highly variable unless due precautions are taken in the separation of the plasma.

It is not clear to what extent, if any, metabolites of CyA contribute to efficacy or to toxicity and accordingly some laboratories also report whole blood or plasma CyA concentrations using a specific HPLC assay. Apparently no laboratories have quoted unbound concentrations of CyA, yet it is generally accepted in pharmacology that response in determined by the unbound drug concentration which is, in the case of CyA, at least an order of magnitude lower than the plasma concentration, because of its binding to plasma proteins [10].

A major objective of this study was therefore to examine in detail the intra- and inter-subject variability in the distribution of CyA within blood and its

binding to plasma proteins in renal-transplant patients receiving the drug both intravenous and orally and, if possible, to account for any observed variability.

Various values for the terminal half-life of CyA have been reported ranging from 2.5 h [11] to 53.4 h [12] and, as half-life determines the time necessary to achieve steady-state for the drug in the body during constant rate administration, a further objective of the study was to determine the pharmacokinetics of CyA during a 3-day continuous intravenous infusion. Additional aims were to estimate oral bioavailability, using the intravenous data as reference and to compare the RIA and HPLC concentration measurements.

Materials and Methods

Clinical

The study received the approval of an ethics committee and all patients gave their informed consent. The study was conducted in 5 patients in all of whom the pharmacokinetics of CyA was examined in detail over a 10-day period.

Intravenous Studies. A constant rate infusion of CyA (7 $mg \cdot kg^{-1} \cdot day^{-1}$) was given via a central venous line over a period of 72 h. The infusion solution was prepared by diluting the commercially available vials [4-7] of CyA into 1 l of sterile saline and was given at a constant infusion rate of between 10.5 and 13.0 $ml \cdot h^{-1}$ using a high-precision infusion pump (IMED 365). The total volume of fluid infused did not exceed 1 l.

The dosing was initiated between 6 and 12 h post-transplant in patients in whom a good diuresis was established. The infusion rate was monitored by periodic observations of the change in weight of the infusion bag.

Timed venous blood samples (12 ml) were taken into EDTA tubes (as anticoagulant) before and at 2, 4, 8, 12, 18, 24, 36, 48, 51, 54, 57, 60 and 72 h after commencing the infusion and were processed immediately or stored at 4 °C for not more than 12 h before processing.

Oral Studies. Each patient was transferred to the oral dose study at 17 mg CyA/kg per day after cessation of the intravenous study. The CyA dose was split and given twice-daily (at 9 a.m. and 9 p.m.) and was dosed as a chocolate emulsion drink in Caotina (Wander AG, Bern). This emulsion was made by absorbing a pre-weighed portion of the commercial CyA dosing solution in olive oil (Sandoz) into the contents of a packet of Caotina in a 100-ml bottle. Cold milk (≈ 80 ml) was added and the mixture shaken vigorously to obtain the emulsion which was then drunk immediately by the patient from the bottle, by means of a straw to ensure complete ingestion of the dose.

Samples of blood were taken into EDTA anticoagulant immediately pre-dose on each dosing occasion, and a 12-h oral profile was obtained during one of the later dosing intervals, usually Days 3 or 4 after initiating the oral regimen, to obtain an assessment of the absolute bioavailability.

Blood samples were taken for the oral profile immediately predose and at 1, 2, 4, 8 and 12 h postdose.

All samples were handled in the same way as the intravenous study samples.

Each patient was monitored carefully for graft status, renal function, clinical chemistry, haematology and concomitant drug therapy.

Blood/Plasma Ratio and Fraction Unbound

The partitioning of CyA into erythrocytes was carried out at 37 °C using tritiated CyA tracer (Batch no. RA 574-1, diluted to give a specific activity of 4000 $dpm \cdot ng^{-1}$), supplied by courtesy of Sandoz Ltd. (Basel, Switzerland).

A 5.0-µl aliquot of radiolabelled CyA (300 ng) dissolved in methanol was freshly spiked into a clean tube and dried in a stream of oxygen-free nitrogen. The CyA was equilibrated with 4.0 ml of blood by incubation for at least 20 min in a 37 °C water bath, with intermittent gentle mixing. The sample was then quickly centrifuged in centrifuge buckets which had been preheated to 37 °C and the concentration of radioactivity in 180-µl aliquots of the plasma (C^*_P) determined by liquid scintillation counting in a LKB Rackbeta 1218 counter using RIA Luma (LKB) scintillator. The total (whole blood) concentration of radioactivity (C^*_B) was determined by counting 5.0 µl aliquots of the spiking solution, thus enabling the C^*_B/C^*_P ratio to be calculated. In blood at equilibrium, the ratio C^*_B/C^*_P was taken as the whole blood/plasma concentration ratio of unlabelled CyA.

The remaining plasma, containing radioactive CyA, was used for measurement of the fraction of drug unbound (f_u) at 37 °C using an ultracentrifugation technique [13].

CyA Concentration in Plasma

This was measured by an HPLC method based on that of Carruthers et al. [14]. The plasma is sepa-

rated at 37 °C and 1.0 ml of the sample is extracted with diethyl ether after addition of Cyclosporin D (CyD) as internal standard, and acidification with HCl. The ether extract is then washed with NaOH solution and the extract blown to dryness. The residue is reconstituted in 200 µl of mobile phase and injected into an HPLC system with the following set of conditions:

1. Column: 25 cm × 0.4 cm packed with Zorbax ODS, 5 µ, maintained at 70 °C in a water bath.
2. Mobile phase: Methanol/acetonitrile/water in the volume ratio 35:40:25. Flow rate = 1.5 ml · min^{-1} (Beckman 110 A pump).
3. Detection: 214 nm (Waters 441 Absorbance Detector with Zn lamp).
4. Retention times: CyA = 16 min, CyD = 21 min.

Standard curves (6 points, 0–2000 µg · l^{-1}) were run concurrently with each batch of samples. Samples and standards in each batch were analysed randomly.

The concentration of CyA in a test sample was determined by calculation of the CyA/CyD peak height ratio and reference against a calibration which was calculated as the line of best fit relating the peak height ratios determined for the standards to their known concentrations. Generally, each patient's sample set was assayed on the same occasion.

RIA Assay

These measurements were carried out on blood and plasma samples (separated at 37 °C) using the standard RIA kits supplied by Sandoz Ltd. (Basel).

Triglycerides

Triglycerides were measured by a colorimetric assay [15] both in total plasma and in the top cut of the ultracentrifuge tube, as an indirect measure of very-low-density lipoprotein plus chylomicrons.

Cholesterol

Cholesterol was measured by a colorimetric assay [16] in both total plasma and in the bottom cut of the ultracentrifuge tube as an indirect measure of high- plus low-density lipoprotein.

Data Analysis

The plasma concentration data, derived by HPLC analysis, pertaining to the intravenous infusion were found to be better modelled by a biexponential than a monexponential equation. The following model [17] was fitted to the data using non-linear least squares regression.

$$C_t = \frac{R_0}{V_1}\left[\frac{A'\,(e^{\lambda_1\theta}-1)\,e^{-\lambda_1 t}}{\lambda_1} + \frac{(1-A')\,(e^{\lambda_2\theta}-1)\,e^{-\lambda_2 t}}{\lambda_2}\right] \quad (1)$$

where
C_t = plasma concentration at time t after start of infusion
R_0 = infusion rate
V_1 = initial volume of distribution
A' = fraction of the initial (zero time) plasma concentration associated with the first exponential term, had an intravenous bolus dose been given
λ_1, λ_2 = exponential coefficients or rate constants ($\lambda_1 > \lambda_2$)
θ (during infusion) = t
θ (post infusion) = a constant (the infusion period)

The steady-state concentration (C_{ss}) was calculated from Eq. (1) with $t = \theta = \infty$

Clearance (CL) during the intravenous infusion was calculated from C_{ss} and the infusion rate from the usual relationship [17]:

$$CL = \frac{R_0}{C_{ss}} \quad (2)$$

Other parameters estimated were the volume of distribution at steady-state (V_{ss}) from the relationship [17]:

$$V_{ss} = V_1\left[\frac{A'}{\lambda_1^2} + \frac{(1-A')}{\lambda_2^2}\right]\cdot\left[\frac{A'}{\lambda_1} + \frac{(1-A')}{\lambda_2}\right]^{-2} \quad (3)$$

and the extent of absorption or bioavailability of the oral dose (f), was calculated in the normal manner [17] using:

$$f = \frac{CL * AUC(O, T)}{D} \quad (4)$$

where AUC(O, T) is the area under the plasma CyA concentration-time curve, within a dosing interval (T) at steady-state, estimated from the data using a linear trapezoid approximation, and D is the oral dose.

Results

Clinical

The transplanted kidneys, in all the patients studied, showed good diuresis prior to the start of the infusion studies and renal function remained good over the study period, with only one clinical „rejection episode".

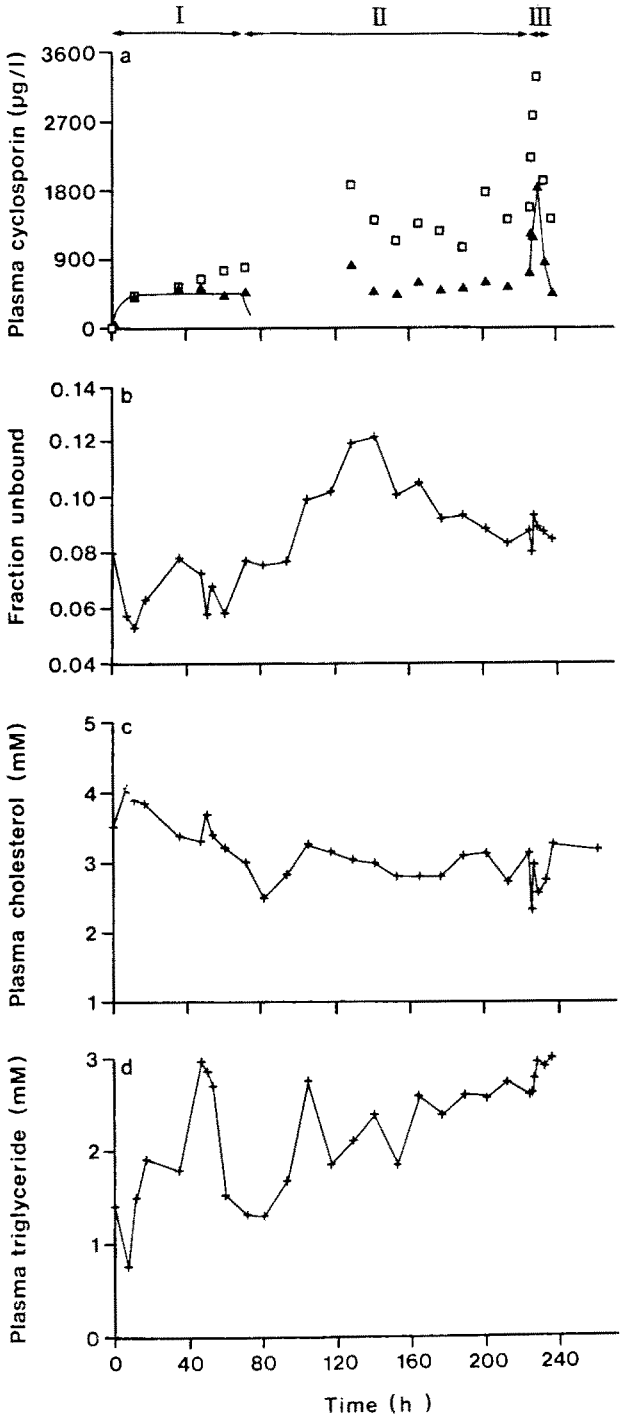

Fig. 1. Changes in measured parameters in Patient 2 during i.v. infusion (I) and oral dosing (II–III): during Period II only trough samples were taken and a complete oral profile was obtained during Period III; **a** plasma cyclosporin concentrations (▲) HPLC (□) RIA; **b** fraction unbound; **c** total plasma cholesterol concentration; **d** total plasma triglyceride concentration

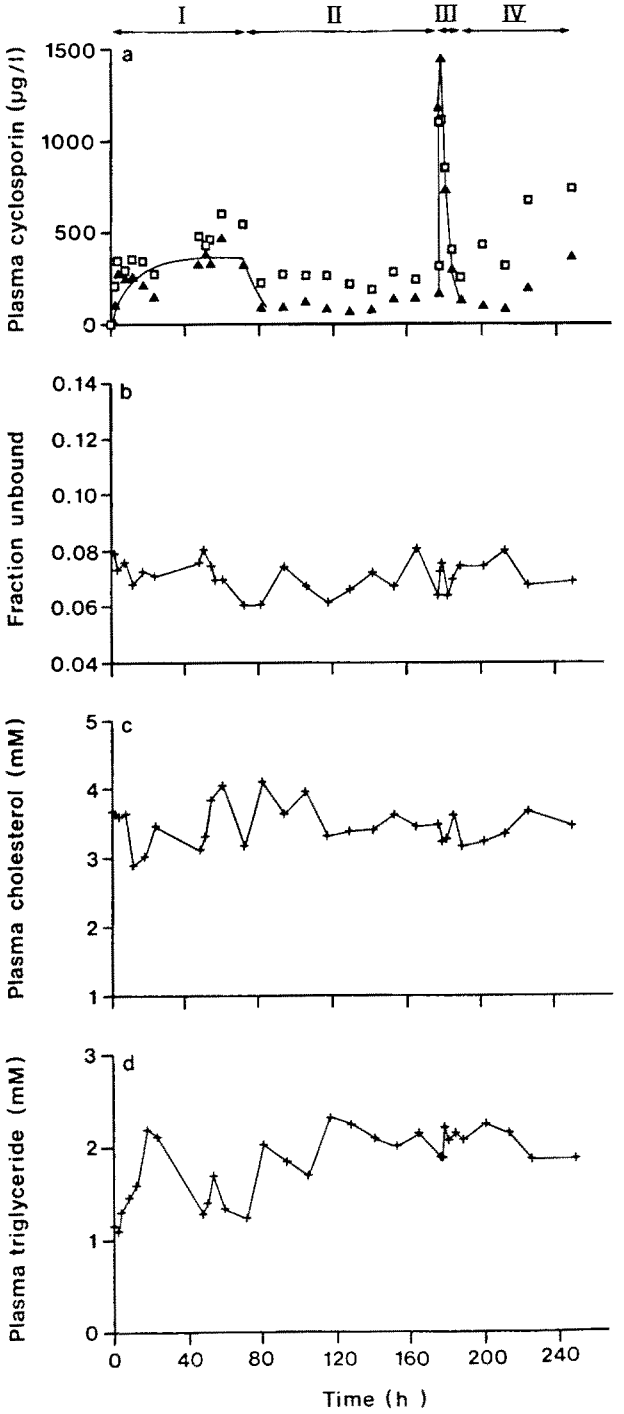

Fig. 2. Changes in measured parameters in Patient 4 during i.v. infusion (I) and oral dosing (II–IV): during Periods II and IV only trough samples were taken and a complete oral profile was obtained during Period III; **a** plasma cyclosporin concentrations (▲) HPLC (□) RIA; **b** fraction unbound; **c** total plasma cholesterol concentration; **d** total plasma triglyceride concentration

The dose of CyA was reduced from 17 to 14 $mg \cdot kg^{-1} \cdot day^{-1}$ in two patients who developed hyperbilirubinaemia during the oral studies.

Plasma CyA Concentration Data

Concentrations of CyA in the plasma samples from the intravenous studies measured by RIA and by

Table 1. Summary of the pharmacokinetic parameters obtained by the fitting of a biexponential function to the intravenous infusion CyA (hplc) plasma concentration-time data

Patient	Weight (kg)	A′	λ_1 (h^{-1})	λ_2 (h^{-1})	V_1 ($l \cdot kg^{-1}$)	V_{ss} ($l \cdot kg^{-1}$)	C_{ss} ($\mu g \cdot l^{-1}$)	$t_{½}$ (λ_1) (h)	$t_{½}(\lambda_2)$ (h)	Clearance ($ml \cdot min^{-1} \cdot kg^{-1}$)
1	71	0.897	0.01	0.098	1.06	3.28	533	0.68	7.1	8.8
2	66	0.950	3.79	0.160	0.358	2.27	459	0.18	4.3	10.3
3	50	0.942	2.23	0.072	0.472	3.56	755	0.31	9.6	6.3
4	59	0.921	5.36	0.087	0.864	7.75	365	0.13	8.0	12.9
5	54	0.946	0.68	0.050	1.61	6.26	447	1.02	13.9	10.6
Mean	-	0.931	-	-	0.873	4.62	512	0.26[a]	7.4[a]	9.8
Range	50-71	-	0.68-5.35	0.05-0.16	0.358-1.61	2.27-7.75	365-755	0.13-1.02	4.3-13.9	6.3-12.9

[a] Harmonic mean

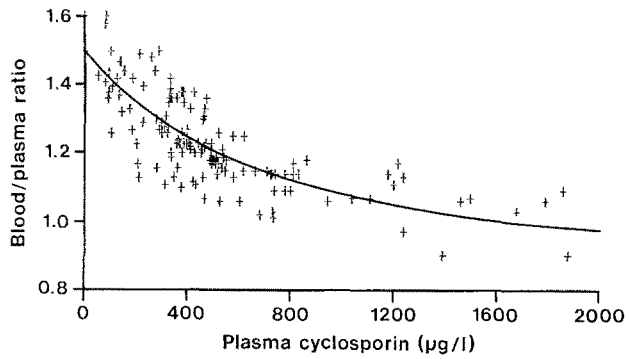

Fig. 3. Variation of cyclosporin blood/plasma ratio with plasma cyclosporin concentration. Combined data for all patients. The solid line is the prediction based on a saturable one-class binding site model of CyA to erythrocyte

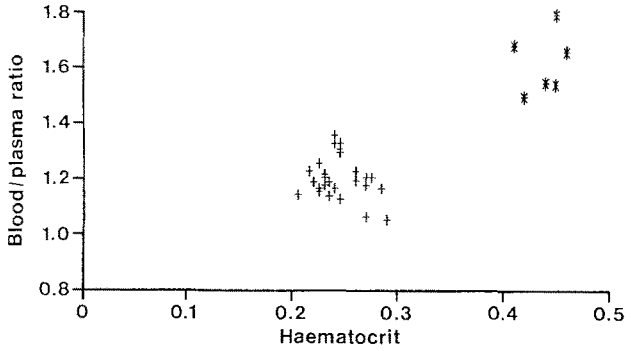

Fig. 4. Influence of haematocrit on the cyclosporin blood/plasma ratio; (+) normal volunteers, (*) patients. All plasma concentrations were in the range 450-550 $\mu g \cdot l^{-1}$

HPLC correlated well, the RIA values generally being slightly higher than the HPLC values.

Correlation of the CyA concentrations in plasma by the two methods, obtained from the oral studies, showed more variability than seen following intravenous administration. Examples of the full CyA plasma concentration data over the 10-day period for two of the subjects are given in Figs. 1 and 2.

During the constant rate infusion, plasma CyA concentration rose progressively with time. Within 18 h of initiating the 72-h infusion a steady-state was reached, using the criterion that the slope of the best least-squares line of the post 18-h points is not significantly different from zero. Thereafter, there was relatively little fluctuation in plasma CyA concentration (e.g. Fig. 1).

Regression of the intravenous infusion plasma concentration-time data, derived by HPLC assay, using the biexponential Eq. (1) gave the parameters presented in Table 1. There was wide variation in λ_1 which varied from 0.68 to 5.36 h^{-1} and in λ_2, with a range of 0.05-0.16 h^{-1}. The corresponding half-life ranges were 0.13-1.02 h and 4.3-13.9 h respectively. The mean central volume of distribution (V_1) was 0.86 $l \cdot kg^{-1}$ and the calculated steady-state concentrations (C_{ss}) ranged over 365-755 $\mu g \cdot l^{-1}$ with a mean of 512 $\mu g \cdot l^{-1}$. The mean clearance calculated was high at 9.8 $ml \cdot min^{-1} \cdot kg^{-1}$. The calculated volume at steady-state (V_{ss}) was, on average, about 5 times larger than the central volume at 4.62 $l \cdot kg^{-1}$.

The oral profiles were obtained during one of the 12-h dosing intervals after steady state had been reached and the maximum concentration (C_{max}), time to reach maximum concentration (t_{max}), AUC and bioavailability (f) for each patient are summarised in Table 2. The absorption was rapid with t_{max} of 2 or 4 h, a C_{max} of 1460-1880 $\mu g \cdot l^{-1}$ with bioavailability estimates of 41-113%.

During the oral study, plasma concentrations of CyA measured by RIA were higher than by HPLC and the blood/plasma ratio was more variable than during the intravenous study as can be seen in Figs. 1 and 2.

Blood-to-plasma ratio measurements of CyA estimated using radiolabelled drug were variable, ranging between 0.90 and 1.76, but correlated well with plasma CyA concentration measured by

Table 2. Summary of the pharmacokinetic parameters obtained after intravenous and oral dosing of cyclosporin to kidney transplant patients

Patient	Haematocrit Range	Fraction CyA Unbound in plasma		Oral dose ($mg \cdot kg^{-1}$)	C_{max} ($\mu g \cdot l$)	t_{max} ($\mu g \cdot l$)	AUC_{oral} ($mg \cdot l^{-1}min$)	Clearance ($ml \cdot min^{-1}kg^{-1}$)	f (%)
		Range	(Mean)						
1	0.24-0.29	0.043-0.074	(0.055)	8.9	1880	4	702	8.8	69
2	0.22-0.30	0.053-0.122	(0.099)	7.4	1860	4	805	10.3	113
3	0.21-0.265	0.042-0.117	(0.068)	9.1	1680	2	594	6.3	41
4	0.21-0.27	0.061-0.081	(0.071)	8.9	1460	2	428	12.9	62
5	0.16-0.25	0.072-0.091	(0.081)	8.9	1790	2	666	10.6	78
Mean	-	0.068		8.6	1730	-	639	9.8	73

HPLC, decreasing with increasing concentration of CyA (Fig. 3). Also, for a given plasma CyA concentration, the blood-to-plasma ratio increased at higher haematocrit values as illustrated in Fig. 4 where ratios in the patients, with low haematocrit (range 0.20-0.30), are compared with those obtained in blood from healthy volunteers, with normal haematocrits (range 0.40-0.46) and CyA plasma concentrations of 450-550 $\mu g \cdot l^{-1}$.

Cyclosporin binds extensively to components within plasma and Table 2 lists the values observed for the fraction CyA unbound in patient plasma. These values varied both among and within individual patients. The mean values for each patient ranged from 0.055 to 0.099. In some patients the fraction unbound varied little (Fig. 2, CV = 8.3%), while in others there was considerable variability with systematic changes with time (Fig. 1).

Triglycerides were measured both in total plasma and in the top fraction of the ultracentrifuge tube. In all the subjects, good linear correlations between the two were obtained ($r^2 = 0.53$-0.76), demonstrating that an estimate of very-low-density lipoprotein plus chylomicrons might be obtained equally well from plasma total triglyceride or from top-fraction triglyceride.

A reasonably good correlation was also found for the cholesterol in the lower sections of the tube and total plasma cholesterol ($r^2 = 0.43$-0.76), demonstrating that either might be used as a measure of low-density plus high-density lipoproteins.

The fraction of the total plasma CyA associated with triglyceride (f_{TG}) in the top fraction of the ultracentrifuge tube was variable and on occasions contained as much as 25% of the total. Good linear correlations were obtained ($r^2 = 0.67$-0.87) when f_{TG} was regressed against the triglyceride content of these sections (Figs. 5 and 6).

The linear correlation of cholesterol, both total and in the section, with fraction unbound in plasma (f_u), both uncorrected and corrected for that CyA associated with triglyceride, was carried out (e.g. Figs. 5c, d and 6c, d). In the patients with sufficient range of cholesterol values the best correlations were obtained with cholesterol in the section vs. f_u corrected ($r^2 = 0.47$-0.69).

Discussion

During the infusion, the CyA concentrations determined by HPLC and RIA correlate well. Knowing that discrepancies as much as 6-fold can occur in these values [14, 18] the close correspondence may be due to either insufficient time for build-up or else to non-production, during intravenous administration, of metabolites which cross-react in the RIA. The small, but noticeable, variation in the CyA concentrations during the 18 to 72-h infusion period were not due to fluctuations in the infusion rate as this was monitored over the whole period and found to be constant. Contributory factors are both assay and biological variability.

The parameters obtained by fitting the intravenous infusion data with the biexponential Eq. (1) are given in Table 1 and show an initial phase with a half-life of 0.13-1.02 h followed by a terminal phase with an associated half-life ranging from 4.3-13.9 h. Highly variable values of terminal half-life were also found by other authors with a range of 3.8-53.4 h [12] and 3.2-9.3 h [27], both groups assaying the drug in whole blood by HPLC.

The volume of distribution at steady-state (V_{ss}) was found to be highly variable and to range from 2.27 to 7.75 $l \cdot kg^{-1}$ similar to the literature values of 1.45-7.26 $l \cdot kg^{-1}$ with a mean of 3.49 ($n = 4$) [28] and 0.12-15.5 $l \cdot kg^{-1}$ with a mean of 4.54 $l \cdot kg^{-1}$ ($n = 41$) (28).

The plasma clearance was found to be high at 6.3-12.9 $ml \cdot min^{-1} \cdot kg^{-1}$ and the average values of 9.8 $ml \cdot min^{-1} \cdot kg^{-1}$ approximates half liver blood

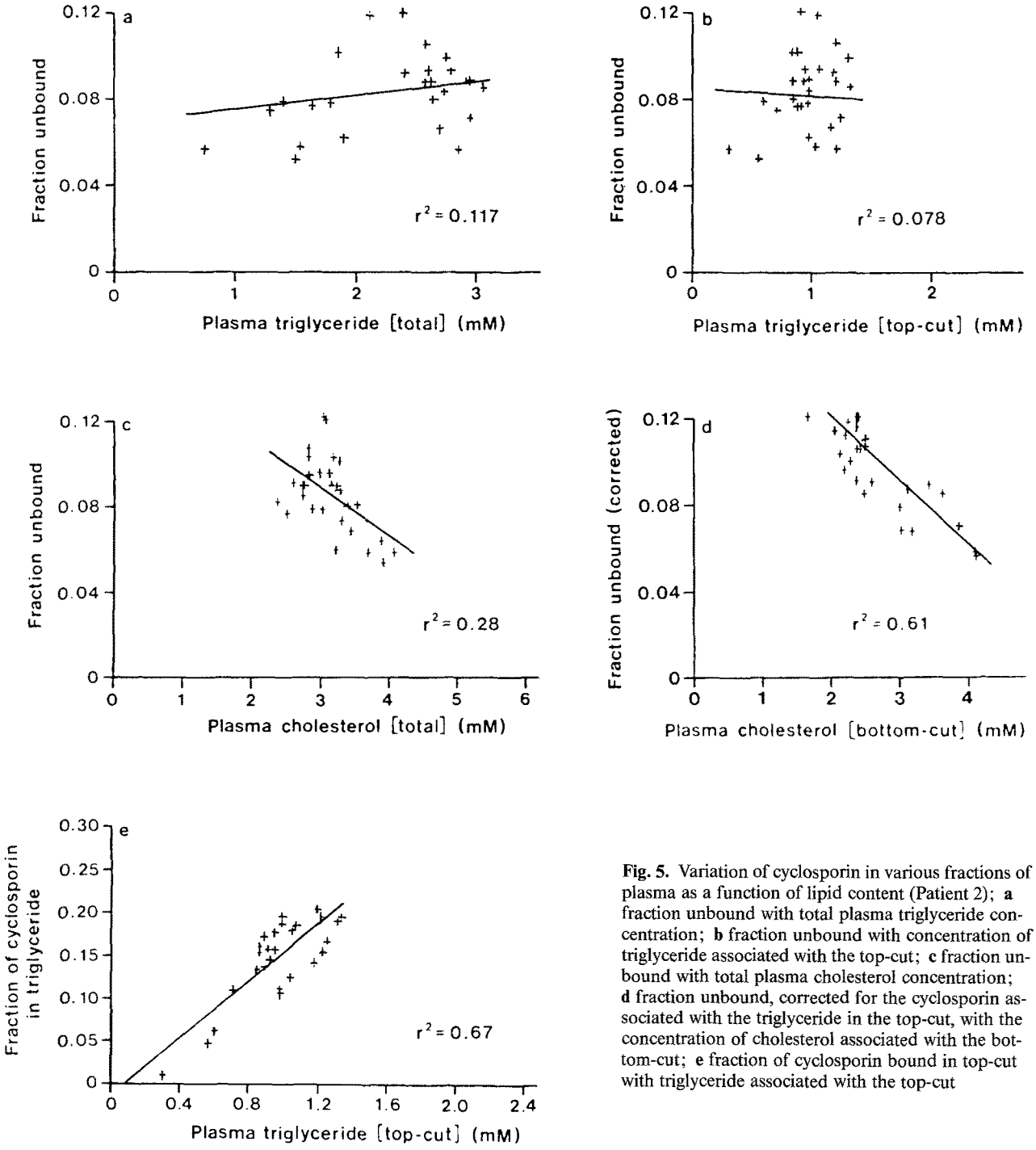

Fig. 5. Variation of cyclosporin in various fractions of plasma as a function of lipid content (Patient 2); **a** fraction unbound with total plasma triglyceride concentration; **b** fraction unbound with concentration of triglyceride associated with the top-cut; **c** fraction unbound with total plasma cholesterol concentration; **d** fraction unbound, corrected for the cyclosporin associated with the triglyceride in the top-cut, with the concentration of cholesterol associated with the bottom-cut; **e** fraction of cyclosporin bound in top-cut with triglyceride associated with the top-cut

flow if a blood/plasma ratio of 1.0 and a liver blood flow of 1500 $ml \cdot min^{-1}$ in a 70 kg man is assumed. As the liver is the major site of elimination of CyA, current models of liver clearance (which assume linear systems) predict that, of that CyA absorbed after an oral dose, >50% would be removed by first-pass through the liver and thus not reach the general circulation. The bioavailability of an oral dose should therefore be <50%.

During the seven day oral studies, the trough levels of CyA, as determined by HPLC, did not show much variation, whereas the RIA determined values fluctuated widely, most probably due to accumulation of metabolites which interfere in the assay.

The bioavailability values of 41–113% found in this study are much higher than generally quoted in the literature but comparisons are difficult because

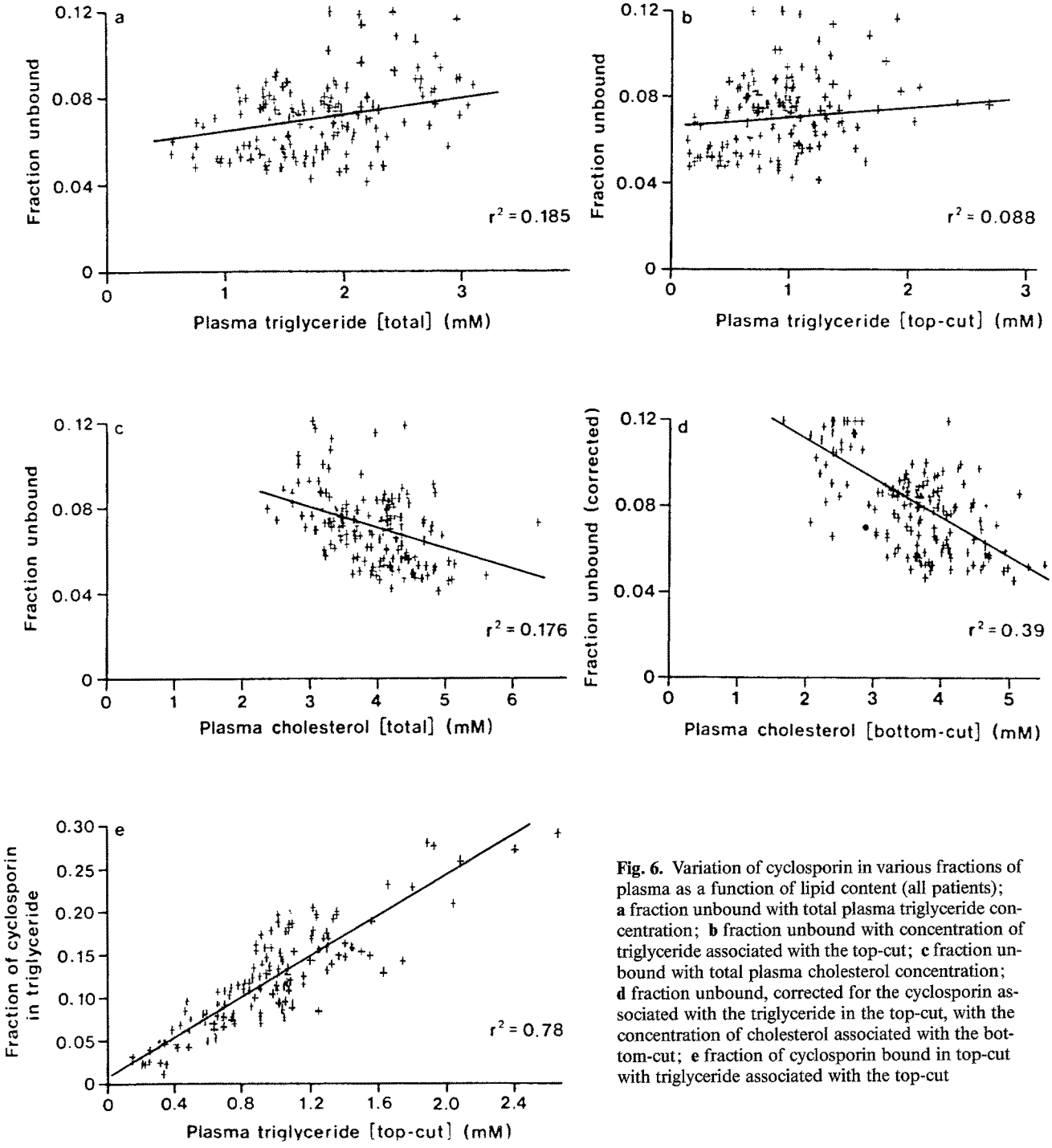

Fig. 6. Variation of cyclosporin in various fractions of plasma as a function of lipid content (all patients); **a** fraction unbound with total plasma triglyceride concentration; **b** fraction unbound with concentration of triglyceride associated with the top-cut; **c** fraction unbound with total plasma cholesterol concentration; **d** fraction unbound, corrected for the cyclosporin associated with the triglyceride in the top-cut, with the concentration of cholesterol associated with the bottom-cut; **e** fraction of cyclosporin bound in top-cut with triglyceride associated with the top-cut

of lack of information given, particularly the techniques associated with the administration of both the intravenous and oral doses. In the studies described here, the oral dose was given in such a way as to ensure complete ingestion of the accurately measured dose, the residual CyA assayed in the dosing bottles showing that >99% of the intended dose was received by the patients. Every attempt was also made to disperse CyA evenly in the chocolate emulsion used in the oral preparation administered.

Literature estimated of bioavailability using RIA analysis are low and variable, 1.1 to 75.3% [19], 4–26% [20] and 2.2–41% [21]. These values may not reflect the true bioavailability because of large errors in the estimation of CyA, introduced from the acknowledged cross-reaction of metabolites in the RIA. As indicated previously this can result in as

much as 6-fold over-estimation of CyA concentration.

Bioavailability estimated from data obtained by HPLC analysis are also variable among patients, <5-19% in paediatric liver transplants [22, 23], < 5-89% in renal transplants [12] and 35 ± 11% in cardiac transplants [22]. Values of 2.2-26.1% were found in liver-transplant patients and such low values may be due to cholestasis or to malabsorption due to incomplete hepatic function, as bile is thought necessary for CyA absorption [23].

It is thought that by analysing CyA specifically in plasma and using the technique described to administer the oral dose, together with the method of continuous intravenous infusion, a technique which does not result in extreme plasma concentrations, has lead to more realistic bioavailability assessments being obtained.

Displayed in Fig. 3 is the variation of the blood/plasma ratio with plasma concentration of CyA (Cp), utilising data from all patients, and illustrates the nonlinear binding of CyA to erythrocytes, which has also been observed in vitro [10]. Scatter is observed in these data as the ratio is also a function of haematocrit (H) and of CyA fraction unbound in plasma (f_u).

On the basis that the therapeutic and toxic effects relate to the parent drug and not to metabolites, then measurement of CyA in plasma water (C_u) is the measurement of choice for therapeutic drug monitoring. If the relationship of C_u to C_p is invariate then measurement of total plasma concentration will be a good index of C_u. However, it is known that CyA binds to plasma proteins, pricipally lipoproteins [24], and that plasma lipoprotein concentrations can vary appreciably even in the normal population [25], thus bringing the above assumption into question. Additionally, some authors conclude that differentiation of nephrotoxicity and rejection by means of CyA levels is not clear-cut [7-9] and for these reasons we measured the fraction unbound in all the patient samples to ascertain its variability. All measurements were carried out at 37 °C as f_u is known to change with temperature [10]. As can be seen in Table 2, values of f_u were observed ranging from 0.042 to 0.122, although most of the values were encompassed in the range 0.05 to 0.08.

The f_u values were found to be relatively constant in three of the patients but systematic changes were seen with time in the other two patients. One of these patients was seen to have a three-fold change in f_u and thus, for the same plasma concentration, a three-fold change in "active" concentration would have existed which may have resulted in the pharmacological or toxicological response moving in an undesired direction.

In anticipation that the variability in f_u might be of sufficient magnitude to question the use of C_p measurements, and the measurement of f_u, using ultracentrifugation, is impractical in a routine clinical laboratory, we examined the relationship of f_u to concentrations of plasma cholesterol and triglyceride (the major constitutents of lipoproteins and thus an index of lipoprotein content) in the hope that f_u could be calculated from these measures. This approach has been used for the binding of amitriptyline and nor-triptyline to lipoproteins in plasma [26].

The measures of triglyceride in the top cut of the ultracentrifuge tube, derived from very low density lipoprotein and chylomicrons, correlated well with the associated fraction of drug (f_{TG}), in all subjects ($r^2 = 0.67$-0.87) and was independent of both total drug concentration and f_u. An example is given in Fig. 5e and for all patients in Fig. 6e. This relationship would result if the drug is considered to undergo a simple physiochemical partition into the triglyceride. In contrast, poor correlations of f_u with both total triglyceride ($r^2 = 0.032$-0.162) and triglyceride in the top cut ($r^2 = 0.028$-0.200) were found as illustrated in Figs. 5a, b and 6a, b. The reason for this is that only a relatively small fraction of the CyA in plasma is associated with the triglyceride.

Additionally, in the three subjects with a sufficiently wide spread of cholesterol values, f_u, corrected upwards for the amount of CyA lost to the triglyceride, when regressed against the cholesterol content in the lower part of the ultracentrifuge tube and which corresponds to the low-density plus high-density lipoprotein, gave correlation coefficients (r^2) of 0.46 to 0.69. Examples are given in Figs. 5c, d and 6c, d. Although these correlations would seem to indicate that some of the variability of binding of CyA is due to triglyceride and cholesterol related materials, the confidence in a predicted f_u value, calculated from measurements of these two lipids species in one plasma sample, will be very low because of considerable variation of individual values. At present, therefore, if estimates of unbouund fraction of CyA in plasma are required, reliance will have to continue on such methods as ultracentrifugation. Monitoring of total plasma triglyceride and particularly cholesterol may, however, be helpful in identifying patients with high or low binding values and patients in which binding is likely to vary widely.

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