

Effect of Hematocrit on Cyclosporine (Cyclosporin A) in Whole Blood and Plasma of Renal-Transplant Patients

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Cyclosporine concentrations in whole blood and plasma were determined in 25 samples from renal-transplant patients. The portion of cyclosporine carried in plasma varied widely, from 9 to 58%. Radioimmunoassay and liquid chromatography both revealed a statistically significant inverse correlation between hematocrit and the plasma fraction of cyclosporine in the circulating blood. Regression analysis indicates that a 10% increase in hematocrit would decrease the portion of cyclosporine in plasma by 12 to 14%. Studies with cyclosporine-supplemented samples further demonstrated an effect of hematocrit on the drug concentration in plasma without a corresponding effect on the concentration in whole blood. The distribution of cyclosporine between plasma and erythrocytes was unaffected by plasma cholesterol and triglycerides for these transplant patients.

Additional Keyphrases: *variation, source of immunosuppressive drugs*

Cyclosporine (cyclosporin A) is a powerful immunosuppressive agent currently used in organ transplantation for prevention of graft rejection. Its therapeutic monitoring has been advocated, because of its nephrotoxic and hepatotoxic effects. The question of whether to monitor blood or plasma, and interpretation of the respective results, have been complicated by the hydrophobic binding of cyclosporine to cellular and dissolved components of blood. Assays for exogenous cyclosporine added to whole blood demonstrate that cyclosporine binds to erythrocytes, leukocytes, and plasma protein with various affinities (1, 2); moreover, this binding depends on the temperature as well as the cellular and lipoprotein content of the sample. Several studies with samples from transplant patients (3, 4) have shown that the ratio of cyclosporine in whole blood to that in plasma varies from patient to patient. This variability may be caused by (a) the temperature dependence of the binding, a factor not easily controlled during plasma collection (2-6), and (b) variability in hematocrit and lipoprotein content, as predicted by *in vitro* studies of cyclosporine supplementation (1). The present study was intended to evaluate the effect of cell volume and plasma lipid content on the distribution of cyclosporine in blood from renal-transplant patients. Because the radioimmunoassay for cyclosporine lacks specificity, I performed a parallel analysis by liquid chromatography and radioimmunoassay.

Materials and Methods

RIA for cyclosporine. For radioimmunoassay of cyclosporine in whole-blood hemolysates and plasma I used the reagents and method from Sandoz Ltd., Basel, Switzerland. Standards were prepared in heparinized blood from healthy volunteers who were not taking any medication. Quench

correction of the liquid scintillation data was by a channel ratio method. The control pools used in each assay were prepared by supplementation of heparinized blood with cyclosporine (Sandoz). The between-run CVs were 7.6% and 10.8% for RIA control pools ($n = 20$) containing an average of 983 and 464 μg of cyclosporine per liter, respectively.

Liquid-chromatographic assay for cyclosporine. For liquid chromatography I modified the method of Carruthers et al. (7), substituting a 4.6 mm \times 25 cm reversed-phase CN column (Du Pont Instruments, Wilmington, DE 19898) for an octyl silica column so as to reduce the column temperature and decrease the organic solvent in the mobile phase. To protect the analytical column, I used a 4.6 mm \times 5 cm guard column packed with Permaphase ETH (Du Pont). The mobile phase, acetonitrile/methanol/water (34/18/48 by vol), was used at a flow rate of 0.75 mL/min. Given the hydrophobicity of the CN stationary phase, a column temperature of 60 °C was adequate. These optimized conditions efficiently separated cyclosporine and cyclosporin D (the internal standard, obtained from Sandoz), with good resolution from potentially interfering peaks. The average retention times for cyclosporine and the internal standard were 13.8 and 16.2 min, respectively. Total chromatography time was less than 17 min.

The drug was extracted from plasma and hemolyzed blood as described in the original method (7). However, I reconstituted the final dried extract in mobile phase instead of ammonium sulfate. The standards prepared for the RIA method were also used in the liquid-chromatographic assay; cyclosporine-supplemented pooled blood was used as a control. Although I used separate control pools in the two assays, the concentration of cyclosporine in the pools was closely matched. The between-run CV for the liquid chromatography pools ($n = 20$) was 3.6% and 5.5% (mean cyclosporine concentrations of 963 and 493 $\mu\text{g/L}$, respectively).

Sample collection from patients. Twenty-five venous blood samples were collected from 21 nonfasting renal-transplant patients who had been taking cyclosporine orally for at least two weeks but no additional medications known to affect cyclosporine metabolism. Blood drawn 4 h after cyclosporine administration was transported at room temperature, equilibrated in a 37 °C incubator by gentle rotation for 2 h, and centrifugated for 5 min at room temperature to obtain plasma. Plasma was obtained within 4 h of blood collection in all cases. After freeze/thaw hemolysis of the blood, both hemolyzed blood and plasma were analyzed by the techniques described above.

Cholesterol and triglyceride analysis. Cholesterol and triglycerides were measured in the 25 plasma samples with an RA-1000 discrete analyzer and methods (Technicon Instrument Corp., Tarrytown, NY 10591).

Cyclosporine supplementation studies. Further to study the effect of hematocrit, I added cyclosporine to medication-free blood samples of various hematocrits. Pooled heparinized blood from healthy nonmedicated volunteers was divided into three aliquots and centrifuged; transferring plasma between aliquots produced hematocrits ranging from 25 to

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51%. After gentle remixing, aliquots were supplemented with 1600 μg of cyclosporine per liter of blood. Temperature equilibration, plasma collection, and cyclosporine analysis was performed as described for samples from transplant patients. Separate pools having a similar hematocrit range were prepared for the RIA and the liquid-chromatographic analysis.

Results

Cyclosporine by RIA and liquid chromatography. The mean concentrations of cyclosporine in whole blood and plasma from transplant patients as determined by RIA were 770 and 379 $\mu\text{g/L}$, respectively. The corresponding mean levels by liquid chromatography were 385 and 235 $\mu\text{g/L}$. The regression equation comparing RIA (y) and chromatographic (x) data was $y = 1.54x + 169$ ($r = 0.91$, $p < 0.001$) for blood and $y = 1.24x + 87$ ($r = 0.79$, $p < 0.001$) for plasma. The regression equation for the comparison of methods for medication-free blood supplemented with increasing amounts of cyclosporine ($y = 1.02x - 3$, $r = 1.00$, $p < 0.001$) did not show the positive slope and y -intercept biases.

Relationship between cyclosporine concentrations in blood and plasma. The ratio of whole-blood to plasma cyclosporine concentrations for the 25 patients' samples as determined by RIA ranged from 1.3 to 6.2 (mean = 2.5, SD = 1.2) vs 1.2 to 2.6 (mean = 1.8, SD = 0.4) by liquid chromatography. As a more direct index of the cyclosporine content of plasma, the percentage of total cyclosporine in whole blood that was found in plasma was calculated from the blood and plasma concentrations of cyclosporine and the hematocrit.¹ By RIA, 30.1% (SD = 13.4%; range 9–58%) of cyclosporine was distributed in plasma; the corresponding finding by chromatographic analysis was 37.4% (SD = 11.1%; range 18–57%). The higher proportion of cyclosporine in plasma as determined by liquid chromatography was statistically significant ($p < 0.05$) by a test for paired data.

Effect of hematocrit and lipids on cyclosporine distribution in blood. Hematocrit data for the patients' blood samples ranged from 24 to 53%, a substantially wider range than the 37 to 49% in a population of healthy males and females studied in our Hematology Laboratory. A regression analysis between hematocrit and the portion of cyclosporine in plasma (Figure 1) showed a significant ($p < 0.001$) negative

correlation by either assay method for cyclosporine, although the slope of the regression equation was more negative for the RIA data. The portion of cyclosporine in plasma decreased by 12 to 14% when the hematocrit increased by 10%. To further evaluate the effect of hematocrit, I repeated the analyses with medication-free bloods supplemented with cyclosporine and analyzed by the methods described previously. As Table 1 shows, the portion of cyclosporine in plasma for these supplemented samples also varied inversely with the hematocrit. Whole-blood concentrations of cyclosporine were, however, unaffected.

The concentrations of lipids in plasma did not affect the distribution of cyclosporine in blood from the transplant patients. Liquid-chromatographic or RIA data for the portion of cyclosporine in plasma showed no significant correlation ($r < 0.20$; $p < 0.60$) with concentrations of cholesterol (range 1.43–4.17 g/L) or triglycerides (0.63–6.28 g/L).

Discussion

The use of plasma or whole blood for monitoring cyclosporine is currently the subject of controversy. Proponents of monitoring whole blood (2–4, 6) point out the effect of temperature on the content of cyclosporine in plasma, the potential for temperature effects during blood transport and processing, and poor correlation between whole-blood and plasma concentrations of cyclosporine. Studies (4, 8) also, however, have shown a poor correlation between concentrations of cyclosporine in plasma and whole blood, even with careful standardization of collection time and temperature. In addition to temperature effects, studies of cyclosporine binding (1, 2) predict an effect of erythrocyte and plasma

Table 1. Effect of Hematocrit on Cyclosporine in Supplemented Whole Blood

Hematocrit ^a	Cyclosporine, $\mu\text{g/L}$		Cyclosporine in plasma, %
	Whole blood	Plasma	
<i>HPLC</i>			
24.5	1586	1067	50.8
39.0	1552	796	31.3
45.0	1608	652	22.3
<i>RIA</i>			
27.0	1581	1269	58.5
37.0	1625	1060	41.1
51.0	1615	601	18.2

^aWhole-blood samples were prepared as described in *Materials and Methods* and were adjusted to give a range of hematocrits.

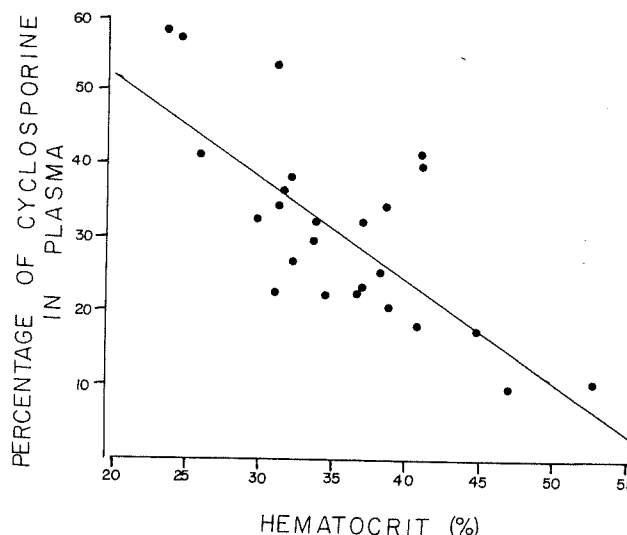
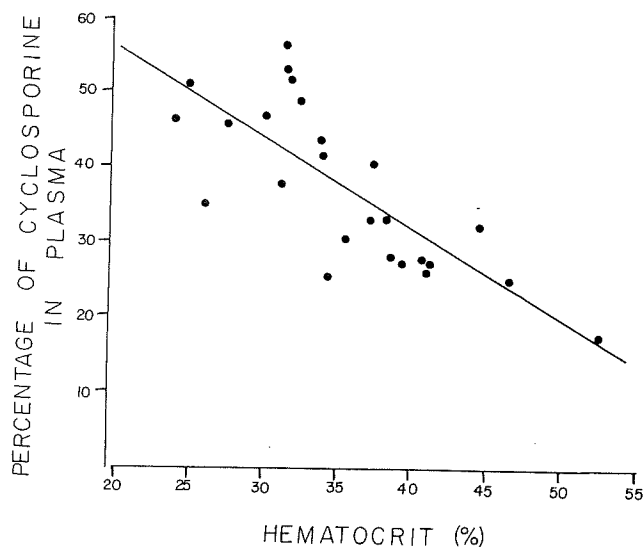


Fig. 1. Correlation of the percentage of cyclosporine in plasma [by HPLC (*left*) or RIA (*right*)] vs hematocrit for renal-transplant patients. The regression lines are: (*left*) $y = -1.22x + 81$ ($r = -0.78$; $p < 0.001$); (*right*) $y = -1.40x + 80$ ($r = -0.73$; $p < 0.001$). $n = 25$ each

lipoprotein content on the concentrations of cyclosporine in clinical samples of plasma. Indeed, a report (8) published during the preparation of this manuscript has shown an effect of hematocrit on plasma content of cyclosporine for post-transplantation patients. My patients' data confirm the effect of hematocrit and further reveals no significant effect of plasma lipids. The average portion of cyclosporine in plasma found in my study compares favorably with the 33% reported by others (1); the range, however, varied widely, in inverse correlation with the variation in blood hematocrit. This effect of hematocrit provides a physiological explanation for the observed discrepancies between concentrations of cyclosporine in plasma and whole blood. A recent clinical report (9) showed an increase in the plasma concentration of cyclosporine following a decrease in the concentration of blood hemoglobin, in agreement with my findings. The increase in cyclosporine concentration was related to signs of nephrotoxicity. In all four clinical cases reported (9) a constant dosage of cyclosporine had been maintained before the toxic episode. A subsequent treatment of the toxicity, by correction of the anemia in two cases or a reduction in the cyclosporine dosage in the other two cases, reversed the nephrotoxic effects; these data suggest that plasma concentrations of cyclosporine correlate well with drug toxicity. Although whole-blood cyclosporine was not measured in their study, results of the cyclosporine supplementation studies here suggest that, when the cyclosporine maintenance dose is unchanged, the whole-blood concentrations of cyclosporine would remain relatively constant despite a hematocrit-dependent fall in the concentrations in plasma. Whole-blood values may therefore not always correlate with plasma findings, as has been confirmed by several investigators.

Other factors may also be important in determining the distribution of cyclosporine within blood. Yatscoff et al. (8) reaffirmed the importance of the conditions of blood transport and temperature re-equilibration; they recommend a 30-min re-equilibration. They also show that an immediate centrifugation at room temperature after the 37 °C re-equilibration has no significant effect on cyclosporine content.

Additionally, as predicted by earlier in-vitro binding studies, the concentrations of plasma lipids may affect cyclosporine distribution in blood (1). Actual patients' data, however, showed no significant correlation of plasma lipids with cyclosporine distribution in blood. This may have been related to the range of concentrations of plasma lipids in the patients studied and does not preclude an effect in more dramatic cases of hyperlipidemia.

Finally, the choice of cyclosporine methodology may affect the apparent drug distribution. In support of previous reports (7, 11-13), I find higher concentrations of cyclosporine in blood and plasma by the RIA than by the liquid-

chromatographic method. This discrepancy has been attributed to cross reactivity of the antibody used with metabolites of cyclosporine. Cyclosporine is now known to be converted to as many as 17 metabolites, nine of which have been identified (10). I have also measured a greater percentage of cyclosporine in plasma when using the liquid-chromatographic method. Perhaps there is a differential binding of cyclosporine and metabolites to cellular and plasma components. Further studies with specific assays for the metabolites will be necessary to determine whether erythrocytes may have a greater binding affinity for the metabolites of cyclosporine.

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