Blood Protein Binding of Cyclosporine in Transplant Patients

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The objective of this study was to compare the binding of cyclosporine to blood proteins between four healthy subjects and five liver and eight renal transplant patients. Fresh heparinized blood was obtained, to which sufficient quantities of tritium-labelled cyclosporine and unlabelled cyclosporine were added to blood samples or red blood cell (RBC) suspensions. Concentrations of cyclosporine in whole blood, plasma, RBC suspension, and phosphate buffer were estimated by liquid scintigraphy. The blood:plasma ratio of cyclosporine in transplant patients was significantly lower ($P < .05$) than that in healthy volunteers. The RBC:buffer ratio, a measure of affinity of RBCs for cyclosporine, was highest in those with liver transplants and lowest in those with kidney transplants. The unbound fraction of cyclosporine in plasma was less in transplant patients than in healthy volunteers. The results of this study indicate that there are differences in blood protein binding of cyclosporine between transplant patients that may contribute to the differences in the pharmacokinetics and pharmacodynamics of this drug.

Plasma protein binding is an important determinant of drug disposition. The binding of drugs to plasma proteins is influenced by a variety of factors.[1] The effect of changes in protein binding on drug kinetics and drug activity depends on the kinetic property and the therapeutic index of the drug. Cyclosporine exhibits marked variability in its kinetics between patients.[2] It is known to be highly bound to plasma proteins in healthy subjects.[3,4] The objective of our study was to compare the binding of cyclosporine to blood proteins between healthy subjects and liver and renal transplant patients.

PATIENTS AND METHODS

Fresh heparinized blood was obtained from eight kidney transplant patients, five liver transplant patients, and four healthy volunteers. All the patients were clinically stable. The plasma unbound fraction of cyclosporine was estimated using a modification of the partitioning method of Garrett and colleagues.[5]

Sufficient quantities of tritium-labelled cyclosporine and unlabelled cyclosporine were added to blood samples or red blood cell suspensions in order to achieve a final concentration of about 300 ng/mL. The red blood cell: plasma distribution ratio was estimated following incubation of blood samples for one hour at 37°C. Distribution equilibrium between blood cells and plasma is observed within 60 minutes.[6] The whole blood was centrifuged at 37°C in order to obtain plasma. Red blood cell to buffer partitioning of cyclosporine was estimated in blood cells, isolated from whole blood, washed three times with isotonic phosphate buffer, and resuspended in phosphate buffer to the initial hematocrit values. Red blood cell suspensions were incubated for 15 minutes at 37°C. Preliminary studies indicated that equilibrium between red blood cells and buffer is rapid and complete within ten minutes.[6] The concentrations of cyclosporine in whole blood, plasma, red blood cell suspension, and buffer were estimated by a liquid scintillation spectrometer using the channels ratio method for quench correction. Whole blood and red blood cell suspensions were counted after bleaching, according to standard techniques.[7] The unbound fraction of cyclosporine in plasma was calculated with the following equation

$$\frac{WB}{P} = \frac{RBC}{B} \times \frac{B}{P}\left[H + (1-H)\right]$$

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in which WB is the concentration of cyclosporine in whole blood, P is the concentration in plasma, RBC is the concentration of drug in red blood cells, B is the concentration of cyclosporine in buffer, H is the hematocrit, and B/P is the unbound fraction of cyclosporine in plasma. A *t* test for independent samples was used to compare different parameters obtained in transplant patients with that of healthy volunteers. A *P* value of less than or equal to .05 was considered to indicate significant differences in the parameters estimated between different groups studied.

RESULTS

The blood:plasma ratio of cyclosporine in kidney and liver transplant patients was significantly ($P < .05$) lower than that in healthy volunteers (Table). Liver and kidney transplant patients also had a lower hematocrit level as compared with healthy subjects ($P < .05$). The RBC:buffer ratio, which is a measure of the affinity of red blood cells for cyclosporine, was very high in all of the subjects studied. The RBC:buffer ratio was highest for the liver transplant group and lowest for the renal transplant group. The mean ± standard deviation RBC:buffer ratio was 13.5 ±3.5, 26.0 ±3.0, and 19.0 ±0.7 in kidney and liver transplant patients and healthy volunteers, respectively. This ratio was fairly constant in liver transplant patients and in healthy subjects but varied more than twofold in renal transplant patients. The unbound fraction of cyclosporine in plasma was significantly smaller ($P < .01$) in liver (0.08) and kidney (0.09) transplant patients as compared with healthy volunteers (0.17). The unbound fraction in plasma ranged from 0.05 to 0.20 in our study population, with considerable variability being observed in the renal transplant patients. The mean unbound fraction in blood was similar to the mean unbound fraction in plasma of renal and liver transplant patients, but smaller in healthy subjects. The unbound fraction in blood varied threefold in renal transplant patients but was less variable among liver transplant patients and healthy subjects.

DISCUSSION

Traditional methods of determining the protein binding of drugs could not be used in the present study. Significant losses of cyclosporine were observed in the equilibrium

TABLE

Blood Protein Binding of Cyclosporine

Subjects	WB/P	RBC/B	B/P	H	B/WB
Kidney Transplant					
1	1.35	18.94	0.11	0.35	0.081
2	1.02	13.42	0.08	0.36	0.078
3	0.85	10.44	0.06	0.43	0.071
4	1.15	8.84	0.16	0.39	0.139
5	0.94	17.25	0.05	0.28	0.053
6	0.93	12.90	0.06	0.37	0.065
7	0.91	15.02	0.04	0.23	0.044
8	1.21	10.95	0.17	0.25	0.140
Mean ± SD	1.05 ± 0.17*	13.47 ± 3.46*	0.09 ± 0.05*	0.33 ± 0.07*	0.084 ± 0.036
Liver Transplant					
1	1.59	29.15	0.10	0.31	0.063
2	1.36	29.60	0.08	0.24	0.059
3	1.27	24.04	0.08	0.31	0.063
4	1.29	24.04	0.08	0.31	0.062
5	1.12	23.55	0.06	0.28	0.054
Mean ± SD	1.33 ± 0.17*	26.08 ± 3.02*	0.08 ± 0.01*	0.29 ± 0.03*	0.060 ± 0.004*
Healthy Subjects					
1	1.69	19.19	0.14	0.43	0.083
2	2.06	19.15	0.20	0.39	0.097
3	2.05	19.60	0.17	0.45	0.083
4	1.93	17.97	0.19	0.39	0.098
Mean ± SD	1.93 ± 0.17	18.98 ± 0.70	0.17 ± 0.03	0.41 ± 0.03	0.090 ± 0.01

**Significantly different from healthy subjects ($P < .05$).*
WB = cyclosporine concentration in whole blood; P = in plasma; RBC = in red blood cells; B = in buffer; H = in hematocrit.

dialysis and ultrafiltration methods, presumably because of binding of cyclosporine to membranes and filters used with these techniques. Ultracentrifugation requires special instrumentation and severe experimental conditions such as high temperature (37°C) and prolonged centrifugation (16 hours) at high speeds (15,000 g).[3] Preliminary ultracentrifuge studies indicated incomplete separation of proteins from plasma water even under such rigorous conditions. We therefore used a partitioning method to study the binding of cyclosporine to plasma proteins. This method yields results that are comparable to that obtained by equilibrium dialysis and ultrafiltration for amitriptyline, imipramine, quinidine, lidocaine, and propranolol.[8] This method also provides information on the erythrocyte uptake of cyclosporine. This method requires that the uptake of drugs into blood cells and the binding of drugs to plasma protein be linear at the concentrations used. Preliminary studies in our laboratory established that the red blood cell uptake of cyclosporine is linear or concentration-independent up to 800 ng/mL. In the present study, we used a concentration of only 300 ng/mL of cyclosporine. Further evidence in the literature indicates that the plasma protein binding of cyclosporine is also linear up to 5,000 ng/mL.[4] Estimations of RBC:buffer and blood:plasma ratios and unbound fractions by this method were very reproducible with a coefficient of variation of 5.3%, 6.8%, and 7.8%, respectively (n = 3).

As shown in the equation, the whole blood:plasma ratio of a drug depends on the hematocrit level, RBC:buffer ratio (RBC uptake), and B/P (unbound fraction in plasma). The wide variation in the whole blood:plasma ratio observed in our study is attributable to the combined effects of differences in hematocrit levels, RBC uptake, and the unbound fraction of cyclosporine in plasma between the different groups. Liver and renal transplant patients often have a low hematocrit concentration as compared with healthy subjects. The fraction of the drug in blood that resides in plasma is inversely correlated with the hematocrit value.[9] Therefore, for a given whole blood concentration, more drug will be distributed in the plasma in these patients. Although cyclosporine was highly sequestered by RBC in all individuals, the uptake was lower in renal transplant patients. The reasons for the poor uptake of cyclosporine by RBC obtained from renal transplant patients is not clear at present, but may be related to qualitative and quantitative differences in the component responsible for drug binding to erythrocytes.

Cyclosporine is primarily bound to lipoproteins in plasma. The large variation in the unbound fraction of cyclosporine in different patients may be a reflection of possible differences in the concentration of lipoproteins in the plasma of these subjects. A high incidence of an abnormal plasma lipid profile has been reported in renal transplant patients.[10,11] Presently, there is no information available on the lipoprotein profiles in liver transplant patients. Lipoprotein profiles were not estimated in our study participants. The contribution of altered lipoprotein concentrations to the variability observed in the unbound fraction of cyclosporine could not be evaluated in the present study.

A combination of altered lipoprotein profile and variable hematocrit will result in marked differences in the unbound fraction of cyclosporine in the blood of transplant patients. Since cyclosporine is a low- to intermediate-clearance drug, its clearance is dependent on its unbound fraction in blood. Wide variations in the unbound fraction will therefore contribute to the wide variability in the clearance of cyclosporine observed in transplant patients. In addition, since hematocrit and lipoprotein concentrations change with time after transplant, time-dependent changes in blood protein binding and clearance of cyclosporine would also be expected.

The results of our study indicate that there are differences in blood protein binding of cyclosporine between transplant patients. This may contribute to the differences in the pharmacokinetics and pharmacodynamics of this immunosuppressant. Future studies should characterize the pharmacokinetics of unbound cyclosporine in blood.

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