Distribution of Cyclosporin A Metabolites among Plasma and Cells in Whole Blood: Effect of Temperature, Hematocrit, and Metabolite Concentration

Gary L. Lensmeyer, Donald A. Wiebe, and Ian H. Carlson

Drug-free whole-blood samples supplemented with the cyclosporines and samples from 10 transplant patients receiving cyclosporin A (CsA) were equilibrated at 4, 22, and 37 °C for 2.5 h; the plasma and cells were separated; and the fractions were assayed by high-performance liquid chromatography (HPLC). Partitioning of CsA and metabolites among plasma and cells was diverse and not always predictable for patients' samples. Overall, although widely variable, >50% of the total concentration of metabolites M1, M8, M9, M10, M16, M17, U1, U8, and U9 in whole blood was associated with the cells; whereas >50% of M13, M18, M21, M25, M26, M203-218, U2, U3, U4, U5, U6, and U7 was associated with plasma. A decrease in hematocrit from 47.8% to 24%, an increase in the sample's temperature (from 4 to 37 °C), or an increase in analyte concentration (usually >500 μg/L for selected metabolites) increased the relative portion associated with plasma in a nonlinear fashion. Parent CsA was most influenced by these changes; its relative concentrations in plasma varied from 18% to 50%. These data support the preferential use of whole blood for therapeutic monitoring of "cyclosporines." Through additional studies, we suggest possible mechanisms affecting the distribution phenomenon and ascribe chemical structure—distribution relationships.

Additional Keyphrases: variation, source of · sample handling · organ transplants · immunosuppressive drugs · chromatography, liquid · metabolism

Therapeutic monitoring of "cyclosporine" has been deemed a useful adjunct to successful immunosuppressive therapy with cyclosporin A (CsA). To be clinically relevant, however, test specimen, processing protocol, and analytical method must be carefully chosen and controlled. Unfortunately, information to guide these choices has been prefaced primarily on results for parent CsA, with minimal attention to the concomitant interactions of the metabolites. CsA is metabolized by liver P-450 cytochrome enzymes to 20 or more compounds; the chemical structures of 12 metabolites have been elucidated (1). Metabolite concentrations are usually greater than parent CsA concentrations in "trough" whole-blood samples and some solid-tissue samples (2, 3). The clinical importance of the metabolites is widely debated, although inhibitory (immunosuppressive) activity—albeit less than observed for parent CsA—has been demonstrated in vivo for metabolites M17, M1, and M21 (4, 5). M17 has been detected in human kidney- and liver-tissue specimens at concentrations greater than parent CsA (3, 6) but has not yet been clearly implicated in the prevalent nephrotoxicity and hepatotoxicity associated with CsA therapy.

Investigators have demonstrated the influences of temperature (7, 8), hematocrit (HCT) (9, 10), and concentration (11, 12) on the distribution of parent CsA among plasma and cells of whole blood. The relative concentration of parent CsA associated with plasma of whole blood decreases when the temperature of a whole-blood specimen cools from 37 °C, or when HCT increases. Various sample-handling protocols have been adopted to control these phenomena. Incubation of whole-blood samples at 37 °C (13, 14), 20 °C (15, 16), or 4 °C (17, 18) before the separation of plasma (or serum) is thought to afford a more representative sample and consistent result. Others report that the distribution is difficult to control and instead suggest the use of whole blood (19, 20). Studies based on use of nonspecific immunoassays to assess the distribution phenomenon further complicate interpretation of analytical results (8, 9, 21). Most immunoassays overestimate parent CsA concentration in patients' samples because of the diverse cross-reactivity of commercial polyclonal antisera with CsA metabolites (22). These inconsistencies and the vagaries associated with metabolite distribution and isolation of plasma/serum from whole-blood samples distort the analytical result.

Few data are available to describe distribution of individual CsA metabolites among plasma and cells of whole blood, due in part to the scarcity of pure metabolite substances for analytical standards and the lack of specific assays for individual metabolites. Earlier, the presence of highly cell-bound metabolite(s) in patients' samples was reported (23); Rosano et al. (5) later identified the compounds as M17 and M1. More recently, Maurer and Lemaire (1) demonstrated diverse distribution patterns for seven metabolites in plasma separated at 37 °C from healthy men given 300 mg of [3H]CsA orally. Whether these data are consistent for plasma separated at other temperatures, for samples with different HCT, and for transplant patients having various pathologies is uncertain.

Here we report the distribution of the metabolites among plasma and cells of whole-blood samples incubated at 4, 22, and 37 °C, and the influence of HCT and analyte concentration on this distribution. We evaluated drug-free whole-blood samples supplemented with authentic metabolites and patients' samples with metabolites deposited in vivo. To separate and quantify individual metabolites, we used solid-phase extraction and HPLC analysis. The practical importance of our results with respect to test sample, handling protocol, and analytical method is discussed. We also propose important interactive mechanisms that affect the observed distributions.

Materials and Methods

Reagents

Chemicals. Acetonitrile was "HPLC" grade from J. T. Baker Chemical Co., Phillipsburg, NJ. Distilled de-ionized water was prepared with the "Milli Q" water purification system (Millipore Corp., Bedford, MA). Solutions of water/
acetonitrile (70/30 by vol) containing CsC (300 µg/L) or M17 (150 µg/L) were used to dilute samples for hemolysis of whole blood and to add internal standards.

Standards: Pure CsA, CsC, and CsD were obtained through the courtesy of Sanofi Pharmaceuticals, East Hanover, NJ. Primary standards of the metabolites M1, M17, M18, M8, M26, M25, M21, M13, M203-218, M16, M10, M9 (chemical structures are listed in Figure 1), and unidentified metabolites (U1 to U9) were purified (from human bile) and standardized as described previously (24). Individual stock solutions (10 mg/L each, in acetonitrile) of CsD, CsC, CsA, and the metabolites were stored at room temperature. M203-218 was unstable and gradually converted to a different form after a few weeks in acetonitrile; we repurified this metabolite before each experiment. To prepare working standards of CsA, C, D, and the metabolites, we pipetted the desired aliquot of stock solution into 12 × 75 mm borosilicate test tubes (Kimax 51; VWR Scientific, Chicago, IL), evaporated the acetonitrile under reduced pressure, and reconstituted the dry standard with a volume of the appropriate matrix to achieve the desired concentration of analyte.

Analytical Methods

We used two assays of "cyclosporine" in our studies:
(a) CYCLO-Trac® (INCSTAR Corp., Stillwater, MN) RIA cyclosporine kit (125I-labeled CsA tracer, polyclonal antibody) was used according to the manufacturer’s instructions. In our laboratory, the between-run precision (CV) was 8.9%, 10.1%, and 6.5% at mean parent CsA concentrations of 91.5, 303, and 983 µg/L, respectively (n = 38 each).

(b) The solid-phase extraction and HPLC procedure were used as described previously (22, 23), with minor modifications, to separate CsD, CsC, CsA, and metabolites. In brief, the cyclosporines are extracted from whole blood, plasma, hemolyzed cells, or water with a Bond-Elut cyanopropyl (CN) sorbent cartridge (Analytichem International, Harbor City, CA) and chromatographed on a Zorbax CN (DuPont Instruments, Wilmington, DE) analytical column with a mobile phase of water/acetonitrile/tetrahydrofuran/acetone/5-ethylamine: 665/375/20/0.3/0.1 (by vol) for CsA/metabolite assay (System A), or 500/500/20/0.3/0.1 (by vol) for the CsA/CsC/CsD assay (System B). Flow rate (0.5 mL/min) and detection at 214 nm were the same for both systems.

Resolution of the cyclosporine in the modified systems is demonstrated in Figure 2. The between-run precision for individual cyclosporines ranged from 7.1% to 9.6% at 200 µg/L.

HCT was determined with the Coulter Counter S Plus 4 (Coulter, Hialeah, FL). The between-run CV for measuring a mean HCT of 40% was 0.93% (n = 1074).

Study Design and Procedure

We carried out the following studies to assess the distribution and interactions of the cyclosporines:

Study A. Cyclosporines added to drug-free whole blood. CsA and nine metabolites (M1, M8, M17, M18, M13, M25, M26, M21, and M203-218) were added, combined and individually, in dry form (200 µg/L each) to drug-free heparinized whole-blood samples from healthy individuals; HCT was 24%, 36.5%, and 47.8%. Each sample was incubated at 37 °C for 1 h to aid dissolution, then divided among a set of three 12 × 75 mm test tubes. Each tube of the set was incubated at 4, 22, or 37 °C, respectively, for 2.5 h, with gentle mixing every 15 min. Samples were then centrifuged (5 min, 1600 × g) in carriers equilibrated to the respective temperature. The plasma and cells were immediately separated and assayed by HPLC. Similarly, we quantified M9, M10, M16, U9, and CsA (200 µg/L each) in whole-blood samples (HCT 27% and 41.5%) and CsA, C, and D (150 µg/L each) in whole blood (HCT 35.0%). Also, unidentified metabolites U1, U2, U3, U4, U5, U6, U7, and U8 (see Figure 2 for retention times by HPLC), which cross-react in the RIA, were added to whole-blood samples (HCT 47%) at 100 to 250 µg/L, incubated at 37 °C, and assayed.

Study B. Cyclosporines deposited in vivo in patients' whole blood. Heparinized whole-blood samples collected before the next dose of CsA from 10 patients with transplanted organs were each divided among a set of three test tubes: incubated at 4, 22, or 37 °C for 2.5 h; then centrifuged, separated, and analyzed for CsA and metabolites as described in Study A.

Study C. Relative distribution of cyclosporines in blood over time at room temperature. We assessed at various times the progress of distribution for CsA (250 µg/L) and metabolites M1, M17, M8, M26, M25, M203-218, M18, M21, and M13 (200 µg/L each) combined in a drug-free heparinized whole-blood sample (20 mL) incubated at room temperature (22 °C) for 4 h. The composite sample was initially incubated at 37 °C for 3 h, then brought to room temperature. Two milliliters of well-mixed blood was removed from the stock sample at 0, 15, 30, 60, 120, 180, and 240 min; centrifuged; separated; and assayed by HPLC and RIA.

Study D. Influence of CsA/metabolite concentration on metabolite distribution. We added CsA and metabolites M17, M1, M18, and M8 individually to drug-free whole-blood (HCT 39%) specimens at concentrations of 0 to 1000 µg/L. Samples were incubated at 37 °C for 2.5 h and processed as in Study A.

Study E. Temperature-dependent solubility of cyclosporines in water. To three 12 × 75 mm borosilicate test tubes we added a mixture of CsA, C, D, M17, M1, M21, and M26 (1 µg each) in acetonitrile, then evaporated the solvent completely. The contents of each tube were reconstituted with 2 mL of Milli-Q-purified water equilibrated at 4, 22, or 37 °C, respectively, then incubated at the same respective temperature and mixed at 30-min intervals. After 2.5 h, a 1-mL aliquot from each tube was combined with 2 mL of diluting solution [acetonitrile/water (30/70 by vol) with appropriate internal standard] and assayed by HPLC.

Fig. 1. Chemical structures of the cyclosporines

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Results

Study A. Distribution of CsA and nine metabolites between the plasma and cells of whole blood at three temperatures is demonstrated in Figure 3A. The concentrations of M203-218 and M13 in plasma remain unchanged from 37°C to 4°C, but the concentrations of M18, M26, M21, CsA, M1, M8, and M17 decreased by 19%, 17%, 8%, 44%, 30%, 31%, and 30%, respectively. These variations in distribution were nonlinear and nonproportional to the incremental adjustments in temperature of the samples.

Also, as HCT varied from 24% to 47.8%, the CsA metabolite concentration in plasma decreased (Figure 3B). The decreases in the concentration of M1, M8, M13, M17, M18, M21, M25, M26, M203-218, and CsA in plasma were 81%, 79%, 45%, 79%, 16%, 37%, 18%, 38%, 39%, and 51%, respectively. On a more practical basis, Figure 3C demonstrates the concomitant effects of HCT and temperature, expressed as deviations from a theoretical equal distribution of analyte between plasma and blood cells. The distribution of CsA was the most variable; M17 was least affected. The distribution of CsA and analogs CsC and CsD is displayed in Figure 3D; the proportions of CsC, A, and D associated with plasma at 37°C were 26%, 36%, and 68%, respectively.

In a separate study (data not shown), concentrations of M9, M10, M16, and U9 in plasma decreased by 36%, 37%, 36%, and 29%, respectively, as HCT increased from 27% to 41.5%. As temperature varied from 4 to 37°C, the proportion of total M9, M10, M16, and U9 in whole blood (HCT 41.5%) associated with the plasma portion changed from 18% to 16%, 11% to 17%, 16% to 23%, and 11% to 17%, respectively. In addition, the proportions of unidentified metabolites U1, U2, U3, U4, U5, U6, U7, and U8 associated with plasma at 37°C (HCT 47%) were 37%, 61%, 67%, 56%, 69%, 61%, 75%, and 20%, respectively.

Study B. HPLC chromatograms of whole blood, plasma, and cell extracts from a renal-transplant patient receiving CsA are illustrated in Figure 4. Table 1 lists HCTs and concentrations of CsA and metabolites in whole-blood specimens from 10 transplant patients. The proportions of CsA and M17 associated with plasma in whole blood incubated at 4, 22, and 37°C for these patients are presented in Figure 5A. Patterns of distribution for each patient are similar; however, the relative amount of CsA and M17 associated with plasma at any single temperature varies significantly among the patients' specimens—differences that cannot be attributed solely to difference in HCT. In Figure 5B we compare distribution data from these 10 patients' samples with data from the drug-free whole-blood specimens supplemented in vitro with the cyclosporines. The similarity of the mean (± SD) relative concentrations of CsA, M1, M8, and M17 associated with the patients' plasma and the mean value for metabolites supplemented into drug-free whole blood supports the validity of the studies in which the cyclosporines were added in vitro.

Fig. 2. Chromatographic resolution of (A) CsC, CsA, CsD, and M17 (internal standard) with system A mobile phase, and (B) metabolites of CsA and degradation product of M203-218 (inset) with system B mobile phase. Concentrations range from >0.1 to 0.18 μg. Unidentified metabolites that cross-react with the antibody in the CYCLO-Trac RIA are denoted with the prefix "U"
Fig. 3. Distribution of CsA and nine metabolites among plasma and blood cells of whole blood: influence of (A) temperature; (B) hematocrit; (C) concomitant effects of temperature and hematocrit; (D) composition of amino acid 2, as represented by CsC, CsA, and CsD
All data are shown as the mean value (n = 3), with CV <10%

Fig. 4. Typical chromatograms of extracts from whole blood (top curve), plasma (middle), and blood cells (lower curve)—separated at 37 °C—from a renal transplant patient just before next dose of CsA
Study C. Changes in plasma concentrations of CsA and metabolites during 4 h at room temperature (22 °C) are illustrated in Figure 6. Each compound reaches equilibrium at a different rate: M17 and M1 slowly re-equilibrate back into the plasma after 60 min, possibly due to displacement of one compound by another on the cells. Fabre et al. (25) described similar observations for the metabolites interacting with lymphocytes. Data from the nonspecific polyclonal RIA are included in Figure 6 to illustrate that the assay is unsuitable for investigating the distribution of CsA metabolites in patients' whole-blood specimens.

Study D. Figure 7 illustrates the influence of analyte concentration on the distribution of CsA, M8, M18, M17, and M1 among plasma and cells. The relative proportion of M18 in plasma remained unchanged from 0 to 1500 μg/L; relative concentrations of CsA, M8, M17, and M1 in plasma increased when their concentrations in whole blood exceed 500 to 1000 μg/L; parent CsA was most affected by concentration.

Study E. Temperature-dependent solubility of various cyclosporines in water is illustrated in Figure 8. Overall, CsA was most soluble at 4 °C (~70%) and least soluble at 37 °C (~40%). The pattern of solubility for CsC and CsD paralleled that of CsA; however, CsE was slightly more soluble and CsD slightly less soluble at all three temperatures. M21 displayed a pattern like CsA but was more soluble (~80%) at 4 °C, whereas M17 and M26 were most soluble at 37 °C (~100%); M1 was equally soluble (~95%) at all three temperatures.

Discussion

Technical Considerations

Experimental conditions were found to be critical to the accurate assessment of CsA/metabolites distribution among plasma and blood cells. In preliminary studies, we manipulated relative proportions of plasma cells to artificially prepare whole-blood samples having various HCT; unfortunately, distributions of the cyclosporines in these distorted samples were not reproducible or consistent with results from unmodified samples with identical HCT. Also, to minimize disruption of the native blood matrix, we supplemented drug-free whole-blood specimens with cyclosporines in dry form; recovery studies confirmed that dissolution was complete.

A precision study was performed to verify that the equilibration-separation technique used to obtain plasma and cells contributed minimally to the diverse results observed in our studies. CsA (250 μg/L) and M18 (150 μg/L) added separately to drug-free heparinized and EDTA-treated

| Table 1: Concentrations of Cyclosporines in Whole-Blood Specimens from 10 Patients |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Patient | HCT, % | M8 | M17 | M18 | M1 | M21 | CsA |
| 1 | 30.5 | 40 | 125 | — | — | 47 | 116 |
| 2 | 35.0 | 37 | 76 | 57 | — | — | 387 |
| 3 | 32.5 | 154 | 367 | — | — | 137 | 260 |
| 4 | 33.0 | 60 | 240 | — | — | 148 | 10 | 249 |
| 5 | 52.0 | 105 | 374 | 58 | — | — | 10 | 249 |
| 6 | 40.5 | 86 | 366 | — | — | 153 | 10 | 240 |
| 7 | 40.5 | 181 | 630 | 25 | 139 | 288 |
| 8 | 41.5 | 117 | 380 | 14 | 77 | 177 | 159 |
| 9 | 36.0 | 221 | 242 | 14 | 132 | 246 |
| 10 | 41.5 | — | 187 | — | 89 | — | 128 |

Fig. 5. (A) Relative concentration of CsA and M17 in plasma of patients' whole-blood specimens incubated at 4, 22, and 37 °C; (B) comparison of distribution data for CsA, M1, M18, and M8 in patients' whole-blood specimens (--) with data from drug-free whole-blood specimens supplemented (in vitro) with the same compounds (- Δ - Δ) Numbers refer to patients' identification in Table 1

Fig. 6. Changes in relative concentrations of CsA and metabolites associated with plasma during 4-h incubation of a whole-blood specimen at room temperature (22 °C)
whole-blood samples \((n = 10\) each) reproducibly partitioned into the plasma after 2.5 h at 37 °C. For CsA, the mean was 177 μg/L (CV 3.9%); for M18 the mean was 207 μg/L (CV 3.8%). Results were essentially identical with both anticoagulants.

The HPLC procedures used in these studies are modifications of our original method reported previously (23). The relative proportions of mobile-phase components were adjusted and the temperature of the analytical column was increased to gain improved resolution and peak symmetry of the peaks for M8, M9, M6, U3, M25, U4, U6, M17, and U9.

Chemical Structure–Distribution Relationships

Relationships between chemical structure and immunosuppressive activity have been established for several analogs of CsA (26). Likewise, we suggest that chemical structure–distribution relationships exist and that specific epitopes of the CsA molecule account for the diverse affinities of blood cells for CsA and metabolites. Alterations of these epitopes modulate the avidity, perhaps by affecting stereochemical and conformational properties of the molecules. Metabolites that are monohydroxylated on amino acid (AA1) (M17), AA9 (M1), or dihydroxylated on AA1 and AA9 (M8) or AA6 and AA9 (M16), are more highly associated with cells from whole blood than is parent CsA. Metabolites demethylated on AA4 (M21, M25) are less associated than CsA with the cells; however, when both demethylation and dihydroxylation of AA4 and AA9 are present in the same molecule (M9), this association with cells is further decreased. Demethylation may disrupt the environment of AA4 or the overall peptide ring conformation (27). Carboxylation of AA1 (M203-218) or formation of a furan derivative of AA1 (M18, M26) substantially decreases the cellular affinity for these compounds; these modifications may possibly distort the overall folding of AA1 side chain of the rigid B-pleated sheet structure of CsA (27). The cellular affinity for CsC and CsD—analogs structurally similar to CsA, except for the AA2—appears to be influenced by the polarity of AA2: CsC (the most polar) is most associated with cells; CsD (the least polar) is least associated with cells. Relatively speaking, the more polar the AA2, the more likely the compound will be associated with cells rather than plasma.

Mechanisms

Processes that govern the distribution of CsA and metabolites among plasma and cells are complex and not well understood. Temperature directs structural conformation(s) of CsA (28); endogenous components of blood can be similarly influenced. Conformations of CsA and some metabolites induced at 4, 22, and 37 °C have different solubilities. In water, parent CsA is more soluble at 4 °C than at 22 or 37 °C; M21 behaves similarly. In contrast, M17 is most soluble at 37 °C; M1 is equally soluble at all three temperatures. Thus, metabolites with unmodified AA1 and AA9 will likely display the diverse temperature-dependent solubility pattern observed for parent CsA. Temperature-induced conformations, temperature-induced ordering of water molecules around hydrophobic moieties (29), and interactions of the cyclosporines with glass may affect solubilization. These data perhaps explain why more CsA in whole blood is associated with plasma at 4 °C than at 22 °C.

In plasma, parent CsA is lightly associated with lipoproteins, primarily high-density lipoproteins, and transfers readily among the various classes of lipoproteins (30, 31).

Whether the metabolites act similarly is unclear and requires in-depth study. Recently, Agarwal et al. (32) reported a 16-kDa cytosolic erythrocyte protein that binds CsA. They demonstrated an almost linear increase in the binding constant as the temperature of the hemolysate decreased from 37 to 4 °C, and proposed these findings as an understanding.
explanation for the temperature-dependent uptake of CsA by erythrocytes. Our data from intact cells in undistorted whole-blood specimens demonstrate that the temperature-dependent interaction of cells with CsA is not a straight-line plot. Instead, we observed relatively more CsA associated with the cells at 22 °C than at 4 °C; moreover, the data were consistent for patients' samples and for the drug-free samples supplemented with CSAs. Obviously, the affinity of cyclosporin-binding protein for CsA is not the sole interaction affecting the distribution phenomenon. Perhaps cyclophilin (33), calmodulin (34), etc. reported to bind CaA—or other proteins may play a role.

Most interesting, though, are the potential interactions with the hydrophobic phospholipid bilayers of the erythrocyte membrane. In past studies, we detected slight amounts of CsA and metabolites in erythrocytes; however, our experimental design bypassed critical membrane dynamics, and we would be remiss to define the influence of membrane solely with this experiment. The lipid bilayer is thought to be a limiting barrier to the transfer of peptides across the membrane. Past investigations have demonstrated an association between CsA and phospholipid vesicles, cytoplasmic lipid droplets in lymphocytes, and CsA receptors on lymphocytes and suggest significant interaction with the membrane lipid bilayer (35–37). Temperature, cholesterol content, fatty acid composition, chain saturation, and lipid–protein interactions influence the lateral movement and fluidity of the membrane phospholipid bilayers (38–41). The more fluid the bilayer, the more permeable the membrane. The bilayer becomes more ordered, crystalline, and less fluid as (a) temperature decreases from 37 to 4 °C, (b) the ratio of cholesterol/phospholipid in the membrane increases (42), and (c) the glycation of membrane proteins becomes excessive, as seen in diabetes mellitus (43). Functions of the membrane are sensitive to these variations. Interestingly, plasma cholesterol is a major contributing factor to the variability in the unbound fraction of CsA in transplant patients (44, 45), and hypertriglyceridemia has been correlated with unusually high "trough" concentrations of "cyclosporine" in serum (46).

The erythrocyte membrane is a nonstatic gateway for interaction of the CsA and metabolites with cytosolic proteins and is influenced by numerous factors. Pathologies that alter erythrocyte membrane integrity and influence the concentrations of cholesterol, lipoproteins, and other endogenous substances probably modulate the distribution of CsA. Unpredictable variations in distribution observed for some patients' samples with similar HCT may in part be explained by such changes. Overall, interactions of the cyclosporines with plasma and blood cells are subtle, unpredictable, and not easily controlled. Most studies, that assess “binding” to cytosolic proteins in the absence of intact cell membranes or acertain membrane binding via ruptured cell ghosts may circumvent the contribution of potentially critical membrane dynamics.

Conclusion

Our studies demonstrate that the distribution of CsA and its metabolites among plasma and cells of whole blood is diverse and they emphasize the dramatic influence of temperature, HCT, concentration, and incubation time on the process. Overall, at 37 °C, with a HCT range of 24% to 47.8%, the relative concentration of CsA and metabolites in plasma ranges from 5% to 95%. The metabolites that potentially can be detected in blood have the following approximate order of increasing association with plasma: M17 < M1 < M8 < M9 < U9 < M16 < M10 < CsA < M21 < M25 < M26 < M18 < M13 < M203-218. As temperature decreases, a lesser amount of each is detected in plasma; similarly, as concentrations of CsA, M1, M17, or M8 exceed a concentration range of 500 to 1000 μg/L, the relative amount in plasma increases. In both cases, these changes are not proportional to incremental variations in temperature or concentration.

These data have practical applications to the selection of specimen, processing protocol, and test method and strongly indicate that current specimen-processing protocols for plasma, although reproducible in results obtained for a single specimen, are inappropriate. Relative distributions of the cyclosporins were not predictable for all patients’ samples. HCT and relative distribution correlated poorly (r = 0.219), and there was a wide range of HCT values in the transplant population as well as marked differences in the HCT of an individual patient during post-transplant hospitalization. Although the influence of temperature, HCT, and CsA/metabolite concentration can be quantified, no algorithm can be established that will account for the concomitant effects. Likewise, laboratories that assay plasma with polyspecific immunosassays risk distortion of the analytical results due to metabolite cross-reactivity and to diversity in the relative concentrations of cyclosporins deposited in blood, in addition to the inconsistencies of metabolite distribution into plasma.

Chemical structure–distribution relationships can be assigned to describe the diverse affinity of the cells for CsA and metabolites. Cells have a greater affinity for metabolites hydroxylated on AA1, AA9, or AA6 than for parent CsA, and are even less avid for metabolites demethylated on AA4, with a furan derivative on AA1, or carboxylated on AA1. Additionally, we infer that the erythrocyte membrane, lipoproteins, cholesterol, and possibly other endogenous substances—widely manipulated by existing pathologies—influence the distribution.

Future investigations need to focus on interactions of the cyclosporines with specific blood components. Defining the role of existing pathologies in modulating pharmacodynamics and distribution of the "cyclosporines" is also important. Unfortunately, the distribution phenomenon is difficult to control, and our understanding of the process remains limited. Sufficient data have emerged, however, to support the use of whole-blood specimens in therapeutic drug monitoring to obviate inaccuracies associated with plasma (or serum) specimens.

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References