Relationships of Cyclosporine Concentrations in Serum, Whole Blood, and Bile after Renal and Hepatic Transplantation

William Vine,1,2 M. Wayne Flye,3 and Peter Jatlow1

"Trough" (minimum inter-dose) cyclosporine concentrations were measured by liquid chromatography in samples of serum and whole blood or bile obtained from renal- and hepatic-transplant patients. Overall, concentrations in whole blood correlated poorly with concentrations in concurrently obtained serum. The poor correlation also held for individual patients over time. The degree of variability observed for individuals is especially disconcerting. Although cyclosporine measurements in whole blood may mitigate time- and temperature-dependent changes in the drug's distribution after collection, concentrations in serum separated after distribution are less dependent on the cellular mass in blood, and may better reflect the amount of drug available to receptor sites. This consideration may be particularly important in the postoperative period, when fluctuations in the cellular mass of blood are frequent. Concentrations of cyclosporines were also determined in concurrently collected bile and serum samples after liver transplantation. Concentrations of unchanged drug in bile were variably higher than those in serum. Bile/serum concentration ratios ranged from 65/1 to 4/61. It is postulated that bile/blood concentration ratios may reflect liver function.

Additional Keyphrases: chromatography, liquid · variation, source of · cellular mass in blood · drug receptors · monitoring therapy

Cyclosporine is a cyclic peptide immunosuppressant that has revolutionized organ transplantation (1, 2). However, its renal, hepatic, and neural toxicity, complicated by a low therapeutic index and variable absorption and metabolism (3, 4), mandates the therapeutic monitoring of cyclosporine, particularly during the initial period of therapy, and requires a better understanding of its hepatic metabolism and enterohepatic circulation. Early procedures for therapeutic monitoring of cyclosporine utilized radioimmunoassay (RIA), which is significantly affected by (presumably) inactive metabolites (4, 5). Recently, we and others (5–8) have developed assays based on liquid chromatography to ensure the specificity that is lacking in RIA.

Because both whole blood and serum can be assayed, the controversy (9–11) of which is preferable for therapeutic drug monitoring can be examined. This issue arose because cyclosporine partitions in a complex, temperature-dependent fashion among erythrocytes, leukocytes, plasma proteins, lipids, and free water (12–14). Therefore, its concentration in serum depends on the temperature of separation and the time to equilibrium, whereas the concentration in whole blood depends on cellular (erythrocyte and leukocyte) mass in addition to the drug's concentration in plasma (11).

If cyclosporine concentrations in plasma and blood were found to be consistently related under suitable conditions of equilibrium, the problem of what is the better sample would be simplified.

Cyclosporine also can be assayed in bile; thus hepatic function, at least as reflected by biliary excretion of unchanged drug, can be assessed. Because more than 90% of cyclosporine is metabolized in and excreted by the liver (1) and because most transplant recipients receive this drug, it might be a good "intrinsic" index of hepatic function after hepatic transplantation. Many hepatic transplant recipients have a decompressive biliary "T-tube" in place, from which bile samples can be easily obtained. We used these samples from several patients to investigate the utility of biliary concentrations of cyclosporine and of bile/serum concentration ratios after liver transplantation.

Methods and Materials

Patients

Patients were recipients of kidney or liver transplants in the interval January to June 1984. Cyclosporine was administered intravenously to liver-transplant recipients and either orally or intravenously to the kidney-transplant recipients, once or twice a day during this study.

Materials

All chemicals were reagent grade or "HPLC" grade. Hexane, acetonitrile, and methanol (HPLC grade) were from Burdick Jackson Laboratories, Muskegon, MI, and HPLC-grade diethyl ether containing 20 mL of ethanol per liter was from Baker Chemical Co., Phillipsburg, NJ. Cyclosporin A and D were generous gifts of Sandoz, Inc., Basel, Switzerland. We used a Perkin-Elmer Series 2 chromatograph equipped with an oven, a Rheodyne injector, and a Kratos no. 757 variable-wavelength detector. The 25-cm column contained 5-μm C8 Spherisorb from Alltech Labs, Deerfield, IL.

Procedure

Blood was collected by syringe to obtain heparinized whole blood, heparinized plasma, or serum. Cells in whole blood were lysed by repeated freezing and thawing cycles. Serum and plasma were separated after standing at room temperature (21 ± 1 °C) for longer than 1.5 h, then either promptly analyzed or stored at −20 °C. Bile, collected from a
biliary T-tube, was also stored at -20 °C until assayed. For the internal standard we added 20 μL of a 40 mg/L methanolic solution of cyclosporin D to 1-mL samples of serum, plasma, or whole blood or to 0.5 mL of bile plus 0.5 mL of drug-free plasma. The mixtures were allowed to equilibrate for 15 min, then acidified with 0.18 mol/L HCl; we then added 10 mL of ether and vortex-mixed for three 20-s cycles. The ether layer was removed after centrifugation and washed with 2 mL of a 0.1 mol/L solution of NaOH in 0.15 mol/L NaCl. After centrifugation the ether phase was dehydrated over anhydrous Na₂SO₄, then evaporated at 37 °C under a stream of nitrogen. We dissolved the residue in 200 μL of methanol, then added 100 μL of water and 2 mL of hexane, mixing after each addition. The hexane layer was discarded and, after a second wash with hexane, we chromatographed 100 μL of the aqueous methanol layer at 60 °C. The mobile phase was acetonitrile/methanol/water (64/14/27 by vol), the flow rate 1.5 mL/min, the detector setting 205 nm, and the recorder setting 0.01 A full scale.

Standards in plasma were freshly prepared on the day of analysis by adding 20 μL of a 200 mg/L solution of cyclosporine to 2 mL of plasma and diluting appropriately with plasma to yield concentrations of 0, 50, 100, 250, and 500 μg/L. A positive 100 μg/L serum-based control was stored frozen in aliquots at -20 °C and analyzed with each assay. The standard curve was prepared by using peak-height ratios of cyclosporin A to cyclosporin D.

Results and Discussion

We have used this method to assay cyclosporine daily for more than 1.5 years for therapeutic monitoring of patients after renal, hepatic, and cardiac transplantation. The detection limit (less than 20 μg/L, defined as 10-fold the baseline noise), reproducibility (CV 4% at 110 ng/mL, n = 10, for between-day analysis), and a standard curve that is linear from 0 to at least 1000 μg/L were more than adequate for these studies. Interfering peaks in cyclosporine-free samples and interferences from other drugs have not been observed. The present method involves features common to several published procedures (5, 7, 8) but combines them uniquely.

Important differences between it and those previously published include a more extensive cleanup and the use of a C₄ column, which together permit rapid elution of cyclosporin A and D in less than 8 min at the relatively low temperature of 60 °C without interfering, late-eluting lipophilic peaks.

Whole-Blood Studies

A good correlation among results for whole blood, serum, and plasma, after equilibration, would validate the use of either sample for therapeutic monitoring. Linear regression analysis of 32 whole-blood and coincident serum samples from 12 patients yielded an r of 0.670 and a slope of 1.37 (SD 0.3) (Figure 1, right). Restricting the analysis to whole-blood concentrations nearer the therapeutic range resulted in poorer correlations: e.g., for whole-blood concentrations <500 μg/L, r = 0.619, slope = 1.1 (SD 0.3) (Figure 1, middle); for <250 μg/L, r = 0.294, slope 0.7 (SD 0.7) (Figure 1, left). The worsening correlation is worrisome because the recommended therapeutic range in whole blood is 100–300 μg/L (6).

Whole-blood/serum concentration ratios varied both between patients and within individuals. For all patients, ratios ranged from 1.1 to 10.1 and averaged 4.3 ± 1.5 (mean ± SD), changing in individuals over time as shown in Figure 2. For example, one patient had a mean ratio of 4.1 ± 1.0 and a range of 2.9 to 5.2 over a one-month period. To eliminate variability due to temperature changes, we equilibrated the serum specimens for over 1.5 h before separation from the clot. The poor correlation and variability in whole-blood/serum concentration ratios in populations has also been found by others, using RIA (9, 13–15). Our data also indicate that variability in this relationship occurs within individuals as well. This comparison of concentrations in whole blood and serum suggests that the relationship between these and the critical "free" drug concentration is complex. The variable amounts of the various cyclosporine-binding components in whole blood—including erythrocytes, leukocytes containing cyclophillin (16), lipoproteins, and albumin—make unlikely a predictable correlation between whole blood and "free" cyclosporine concentrations.
Other observations also support a preference for assaying serum or plasma samples. Changes in hematocrit affect the distribution of cyclosporine in whole blood (11), and changes in the leukocyte count might be expected to do the same. This effect is important because erythrocyte and leukocyte counts can change widely and rapidly in transplant recipients. Nuberger et al. (17) have described nephrotoxicity associated with a decrease in hemoglobin and an increase in serum cyclosporine concentration in four patients. The selection of plasma or serum may be especially desirable when RIA is used because a cross-reacting metabolite of cyclosporine preferentially binds to erythrocytes (18).

The ultimate goal is a measurement that estimates or reflects the free drug concentration. A possibility could be the drug’s concentration in erythrocytes when normalized for hematocrit. Analysis of whole blood, however, will not accomplish this goal. This point is controversial and the use of whole-blood concentrations has been recommended in several reports (6, 19, 20); however, insufficient information is available to be doctrinaire about this issue. We agree that whole blood is the preferred specimen for pharmacokinetic studies where factors affecting distribution can be avoided and clinical correlation of each measured cyclosporine concentration is not the purpose.

Bile Studies

Because concentrations of cyclosporine in bile may also have clinical relevance after hepatic transplantation, we determined these in several patients. In five patients studied so far, there was a lag time between the intravenous administration of cyclosporine and its appearance in the bile, with peak bile concentrations occurring between 6 and 8 hours after a dose (Figure 3, top) then declining over the next 6 to 8 hours. Patients demonstrated the expected cyclical pattern of peak and “trough” biliary drug concentrations (Figure 3, bottom). Concurrent concentrations of cyclosporine in bile and in “trough” serum were measured over extended periods in four patients. Cyclosporine concentrations ranged from 336 to 3280 μg/L; bile “trough” serum concentration ratios varied from >68/1 to 4.6/1 (Table 1). The ratios seemed to decrease over the hospital course as liver function stabilized. Patient 3 (Table 1) had rapidly decreasing ratios (68 to 16) as hepatic function improved after transplantation.

Table 1. Concentrations in Bile and Bile/Serum Concentration Ratios of Cyclosporine in Hepatic Transplant Recipients

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*Trough bile concentrations of cyclosporine, μg/L.
*Bile/serum concentration ratios of cyclosporine at “trough.”
Several measures of biliary excretion of cyclosporine can be followed, including clearance, concentration, and bile/serum concentration ratio. Concentrations rather than clearances are reported because the biliary samples collected represented variable and unknown fractions of the total bile excretion. Concentrations in body fluids are a function of dose, bioavailability, volume of distribution, and rate of hepatic clearance. Bile/"trough" serum concentration ratios may be relatively independent of dose and bioavailability and depend more upon hepatic function. The significance of the ratio is difficult to assess because acute changes are usually superimposed upon a steady overall improvement in hepatic function, as reflected by decreasing values for bilirubin and aspartate aminotransferase. The lower ratios of unaltered (unmetabolized) cyclosporine, observed during improved graft function, may reflect increasing metabolic clearance with decreased excretion of the parent compound; this could be further evaluated by quantifying metabolites of cyclosporine in bile. In contrast, Venkataramanan et al. (21) reported that impaired function results in a decrease in total cyclosporine excretion; however, the effect they observed may be mostly due to diminished bile production and possibly decreased cyclosporine uptake. Moreover, our data suggest that the sampling time is critical, the biliary concentrations varying widely over the interval between doses. Regardless, both reports indicate that impaired liver function changes hepatic clearance of cyclosporine and that metabolites of cyclosporine should be studied further.

References