685

Cyclosporine Trough Concentration Monitoring in Liver Transplant Patients

G.J. Burckart, R.J. Ptachcinski, R. Venkataramanan, S. Iwatsuki, C. Esquivel, D.H. Van Thiel, and T.E. Starzl

CYCLOSPORINE A (CsA) monitoring of blood or plasma concentration measurements is recognized as an important part of the care of a transplant patient receiving the drug. The quantitation of CsA in biological fluids is essential for several reasons: (1) wide variability in the amount of CsA absorbed and the clearance rate of CsA have been observed, particularly in orthotopic liver transplant (OLT) patients[1]; (2) low CsA concentrations in blood or plasma secondary to poor drug absorption or rapid CsA elimination can be detected and adjusted; these situations are common in OLT patients due to diversion of bile through a T-tube and due to the occasional need for anticonvulsant administration; (3) high blood or plasma concentrations of CsA can be avoided through routine monitoring programs; these high levels may occur in OLT patients secondary to time-dependent increases in CsA absorption or to clamping of the T-tube; drugs that inhibit CsA metabolism such as erythromycin[2] also can complicate therapy in OLT patients; (4) blood or plasma concentration monitoring aids adjustment of CsA dosage during the transition from intravenous (IV) to oral therapy and from a high initial oral dose to a lower long-term maintenance CsA dose; and (5) patient compliance with the medication regimen can be followed by blood or plasma CsA measurement.

CsA can be measured in whole blood or in plasma and serum samples. Important differences exist among the sample matrices and have been discussed previously.[3] The interpretation of assay results from the two analytical methodologies available, high-performance liquid chromatography (HPLC) and the radioimmunoassay (RIA), is particularly critical in OLT patients. This article therefore discusses the appropriate timing of a trough sample and outlines those factors that affect the RIA and HPLC results in OLT patients.

SAMPLE TIMING

The trough concentrations of CsA should be that time at which the blood or plasma concentration is lowest. A delay in the rise of CsA concentrations following oral ingestion occurs because of delayed gastric emptying, but concentrations in blood or plasma increase by 1 hour following an oral dose. In most institutions, trough levels are obtained for drug concentration determination within 1 hour prior to the drug dose.

The dosing interval may determine how critical the timing of a trough CsA concentration should be. With terminal elimination half-lives ranging from 10.7 hours in renal transplant patients[4] to 20.4 hours in patients with hepatic cirrhosis,[5] small differences in sample timing will have little effect on the trough concentration if CsA absorption and distribution are complete. Therefore, no clinically significant difference will occur in trough samples obtained 1 to 2 hours prior to a dose when the patient receives CsA as a once-daily dose. However, shorter dosing intervals of 8 or 12 hours may require more precise timing of trough CsA samples. CsA

From the Schools of Pharmacy and Medicine, University of Pittsburgh, Pittsburgh, PA.

Supported in part by Grant 5R01AM34475, National Institute of Arthritis, Diabetes, Digestive, and Kidney Diseases, and by Sandoz Pharmaceuticals, East Hanover, NJ.

Address reprint request to Dr G.J. Burckart, University of Pittsburgh, Clinical Pharmacokinetics Laboratory, 807 Salk Hall, Pittsburgh, PA 15261.

0041-1345/86/1806-5026$03.00/0

concentrations in blood or plasma may not peak until eight hours after an oral dose,[5] and the absorption and distribution processes are frequently prolonged for up to 12 hours. The change in CsA concentrations in blood or plasma during the interval of 6 to 12 hours following an oral dose is rapid in comparison with the slow elimination half-life of the drug. Altering the timing of the trough sample by 1 to 2 hours in a patient on an 8- to 12-hour CsA dosing schedule can result in significantly different blood or plasma concentrations. For example, Fig 1 presents the blood concentrations of CsA measured by RIA and HPLC following an oral dose in a transplant patient. The CsA blood concentrations measured by HPLC at eight hours (544 ng/mL) fall rapidly to the 12-hour concentration (127 ng/mL). The blood concentrations in this same patient measured by RIA decreased from 1,512 ng/mL to only 1,244 ng/mL, resulting in a significant change in the ratio of RIA:HPLC. Imprecise sampling of blood during the CsA absorptive and distributive phases of this patient could result in differing trough concentrations and their interpretation. Because changes in gastrointestinal (GI) function, dose administered, or concurrent drug therapy influence the absorptive pattern of CsA, precise trough sampling timing in patients on 8- or 12-hour dosing schedules should improve the reproducibility and clinical utility of blood or plasma monitoring for CsA.

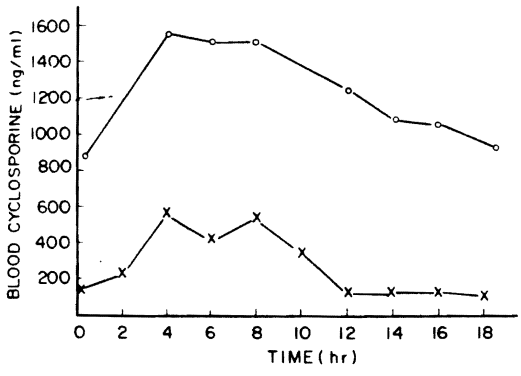


Fig 1. Blood cyclosporine concentrations v time in a transplant patient following an oral drug dose. Measurements are by HPLC (x) and RIA (O).

Transplant patients on multiple daily doses of CsA should have their trough blood or plasma concentration sampled at the same time each day. CsA clearance rates have a diurnal variation with a more rapid clearance rate during the nighttime hours.[6] Predose trough concentrations increased 38% and 72% when evening samples were collected in comparison with morning samples in two OLT patients.[6] Individual patient and transplant center sample times should be standardized so that the center can develop its own consistent guidelines for acceptable desired CsA concentrations. Differences in the sampling time and the CsA dosing interval used may account for the minor variations in the desired CsA range noted between transplant centers (see Table 1).

Changing the CsA dosing interval without a change in total daily CsA dose can have a major effect on blood or plasma concentrations. In a small number of renal transplant patients who received the same daily CsA dose but had their dosing interval changed from every 24 to every 12 to every 8 hours, average steady-state CsA blood concentrations increased from 497 to 800 to 1,241 ng/mL, respectively.[5] Dose-dependent changes in drug absorption or elimination must

Table 1. Desired Trough Cyclosporine Concentrations From Major Transplant Centers

Transplant Population	Biological Fluid	Assay	Desired Cyclosporine Concentrations (ng/mL)
Liver[7]	Whole blood	HPLC	100-400
Renal[8]	Whole blood	HPLC	100-200
Renal[9]	Whole blood	HPLC	150-300
Renal, first month[10]	Plasma	RIA	<500
Renal, long-term[10]	Plasma	RIA	<50-150
Renal[11]	Serum	RIA	100-400
Renal[12]	Serum	RIA	100-500
Renal[13]	Serum	RIA	150-300
Renal[14]	Serum	RIA	100-200
Bone marrow[15]	Serum	RIA	100-250
Heart[16]	Plasma	RIA	100-200
Heart[17]	Serum	RIA	200-500
Liver[18]	Whole blood	RIA	800-1000

be considered when interpreting trough CsA concentrations after a dose or dosing interval alteration.

RIA:HPLC CONCENTRATIONS

Concentrations obtained by RIA analysis are consistently higher than those obtained by HPLC due to the cross-reactivity of CsA metabolites with the antiserum used in the RIA kits.[19,20] The percentage of cross-reactivity of some of the metabolites tested ranges from 4% to 32%. The RIA:HPLC ratio of CsA concentrations in blood, plasma, and serum is highly variable[19,21,22] and depends on the patient's liver function, the time of blood sampling in reference to the time of drug administration, the absolute CsA concentration, and whether the patient is receiving other drugs that alter hepatic metabolizing enzyme function.

Assay results from the RIA should be used with particular caution during periods of impaired hepatic function. In the first 2 weeks following liver transplantation, during liver rejection, or in the event of a technical failure (eg, hepatic artery thrombosis), the RIA:HPLC ratio is dramatically increased.[23] Table 2 presents examples of the peak RIA:HPLC ratios observed early in the postoperative course of ten liver transplant patients. The ratio of trough concentrations measured by the two assays stabilized in the RIA:HPLC ratio of 1.7 to 4.2 if hepatic function was also improving. A similar observation has been made in two OLT patients by Blyden et al,[24] who also report a positive correlation between the total serum bilirubin concentration and the RIA:HPLC ratio. In an earlier report,[23] we also noted that significant increases in the RIA:HPLC ratio to greater than 10 in OLT patients with rises in bilirubin were associated with rejection or a technical complication. Smaller increases in the RIA:HPLC ratio were occasionally associated with increases in liver enzymes without a change in bilirubin.

Patients with severe liver disease have the ability to metabolize CsA as evidenced by their 0.8 to 4.8 mL/min/kg clearance rate[5] but have a significant impairment in their ability to excrete CsA metabolites. CsA blood concentration *v* time data in a patient with liver disease as analyzed by RIA and HPLC are presented in Figure 2. The RIA:HPLC ratio changes over the dosing interval studied (from 1:1 to 1:4), and the pharmacokinetic parameters calculated are different[5] depending on the analytical procedure used.

The time of the trough blood sampling for CsA can significantly affect the RIA:HPLC ratio. In Fig 1, sampling eight hours after the oral CsA dose would have yielded an RIA:HPLC ratio of 2.8, but sampling at 12 hours following the dose would change the results and ratio to 9.8. This difference in the

Table 2. Ratio of RIA:HPLC Trough Blood Concentrations in Ten Liver Transplant Patients

	Early Peak		Stable	
Patient	Ratio	Postoperative Day	Ratio	Postoperative day
1	4.7	3	2.2	17
2	4.8	6	2.0	9
3	5.2	6	1.7	10
4	10.0	4	3.0	13
5	5.4	2	2.7	5
6	4.7	2	2.1	13
7	7.5	2	4.2	7
8	3.3	2	1.7	5
9	5.6	1	2.8	7
10	8.2	6	2.4	9

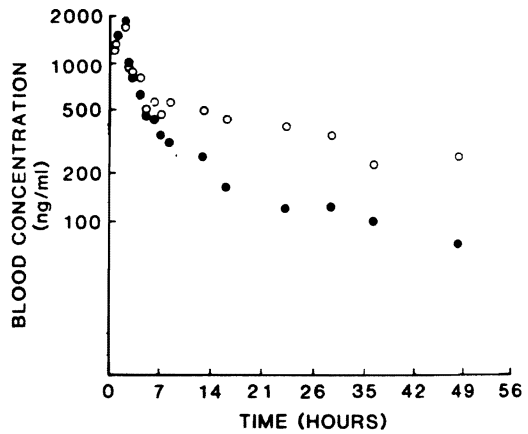
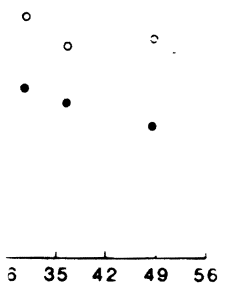


Fig 2. Blood concentrations of cyclosporine *v* time in a liver failure patient following a single IV drug dose. Measurements are by HPLC (●) and RIA (○).

ents by Blyden et al,[24]
ve correlation between
bin concentration and
In an earlier report,[23]
ificant increases in the
eater than 10 in OLT
lirubin were associated
chnical complication.
the RIA:HPLC ratio
iated with increases in
change in bilirubin.
liver disease have the
CsA as evidenced by
in/kg clearance rate[5]
impairment in their
etabolites. CsA blood
ita in a patient with
l by RIA and HPLC
2. The RIA:HPLC
osing interval studied
the pharmacokinetic
re different[5] depend-
edure used.
h blood sampling for
fect the RIA:HPLC
eight hours after the
have yielded an
but sampling at 12
would change the
his difference in the



of cyclosporine v time
a single IV drug dose.
nd RIA (O).

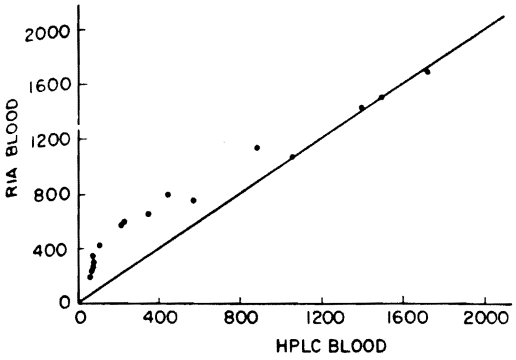


Fig 3. Blood concentrations measured by RIA v HPLC in a single patient over one dosing interval. The solid line represents a 1:1 HPLC:RIA ratio.

RIA:HPLC ratio over a dosing interval is explained by two observations. First, higher concentrations of CsA measured by HPLC result in better agreement between the RIA and HPLC assays because the parent CsA compound then comprises the majority of substance measured by RIA. At lower CsA concentrations in blood or plasma, the RIA is more likely to overestimate CsA, resulting in a higher RIA:HPLC ratio (see Fig 3). Second, the elimination half-life measured by RIA is greater than the CsA elimination half-life measured by HPLC. Therefore, the fall in CsA concentration in blood or plasma during a dosing interval measured by RIA will not parallel the decline measured by HPLC.

Other pharmacologic agents may affect the formation or elimination of CsA metabolites, thereby affecting the RIA:HPLC ratio. For example, previous reports[25,26] have shown that CsA concentrations measured by RIA increase when methylprednisolone is administered concurrently with CsA. Our own observations in patients receiving high-dose methylprednisolone have not demonstrated any difference in CsA clearance measured by HPLC when compared with a control period.[27] When patient trough concentrations were monitored by RIA and HPLC, an increase in the RIA:HPLC ratio was observed coincident with the administration of pulse doses of methylprednisolone (Fig 4). Methylprednisolone affects the metabolism of CsA by enhancing the production of metabolites that react with the RIA but that have little effect on HPLC measurements. The effect of other drugs on the RIA:HPLC ratio is unknown but should be carefully monitored when pharmacologic agents known to alter CsA blood or plasma concentrations are taken concurrently with CsA (see Table 3).

The importance of alterations in the RIA:HPLC ratio will become clearer as the

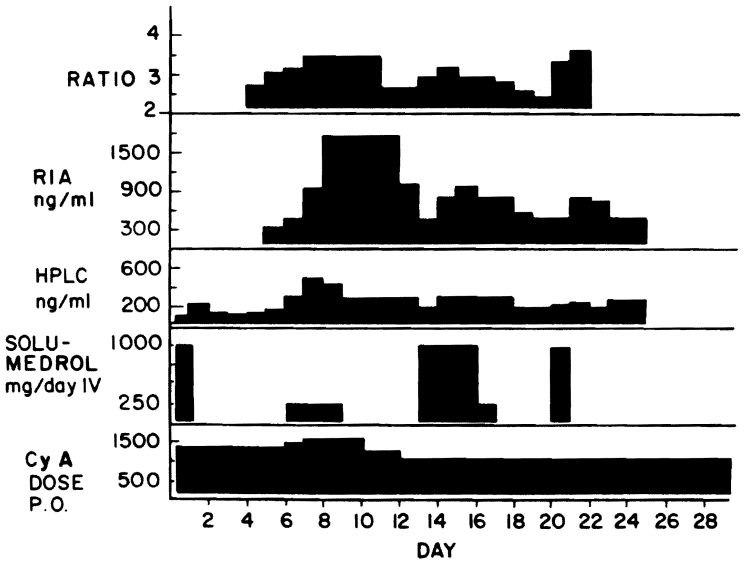


Fig 4. The ratio of RIA:HPLC blood trough concentration measurements in a transplant patient over time. Note the increase in the ratio in association with Solu-Medrol (methylprednisolone) dosing.

Table 3. Drug Interactions With Cyclosporine That Alter Blood or Plasma Measurements

Drug	Mechanism	Effect Observed
Ketoconazole[28-30]	Impairment of cyclosporine metabolism	Increased blood concentrations of cyclosporine leading to nephrotoxicity
Erythromycin[31]	Impairment of cyclosporine metabolism	Increased blood concentrations of cyclosporine
Methylprednisolone (high-dose)[25,26]	Not known	Increased plasma and blood concentrations of cyclosporine as measured by RIA; unchanged blood concentrations of cyclosporine as measured by HPLC; significance unknown
Phenytoin[32,33]	Induces cyclosporine metabolism	Lowering of cyclosporine blood concentrations with associated rejection of transplanted organs
Rifampicin[34,35]	Induces cyclosporine metabolism	Lowering of cyclosporine blood concentrations with associated rejection of transplanted organs
Phenobarbitone[36]	Induces cyclosporine metabolism	Lowering of cyclosporine blood concentrations with associated rejection of transplanted organs
Sulphadimidine-trimethoprim (IV)[37]	Not known	Decrease in serum cyclosporine levels; significance unknown

immunologic and toxicologic effects of CsA metabolites are defined. At present, the metabolites of CsA are considered to have little pharmacologic activity.[38] If this observation is true, RIA trough CsA measurements could be misleading during periods of hepatic dysfunction or when other agents, such as methylprednisolone, alter the RIA:HPLC ratio. Trough CsA concentrations measured by RIA have been associated with both the nephrotoxicity of CsA[15] and the tremors that the drug can induce.[24] The RIA can provide useful trough CsA measurements on OLT patients who have stable hepatic function but should be used in conjunction with HPLC during the immediate postoperative weeks, during periods of changing hepatic function, and when other pharmacologic agents are administered that could alter the metabolism of CsA.

SUMMARY

Trough blood or plasma concentration measurements of CsA must be carefully interpreted in OLT patients in relation to hepatic function, sample timing, assay specificity, and concurrent drug therapy. The RIA:HPLC ratio of blood or plasma measurements will vary with the patient's liver function, the time of blood sampling in reference to the time of drug administration, the absolute CsA concentration, and concurrent use of drugs that may alter the metabolism of CsA. The RIA assay should be used in conjunction with HPLC for trough blood or plasma measurement during the first postoperative weeks, during periods of changing hepatic function, and during changing drug regimens. In the future, the specific measurement of active or toxic metabolites of CsA should improve trough CsA concentration monitoring in OLT patients.

REFERENCES

1. Burckart GJ, Venkataramanan R, Ptachcinski RJ, et al: J Clin Pharmacol (in press)
2. Ptachcinski RJ, Carpenter BJ, Burckart GJ, et al: N Engl J Med 313:1416, 1985
3. Ptachcinski RJ, Burckart GJ, Venkataramanan R: J Clin Pharmacol 26:358, 1986
4. Ptachcinski RJ, Venkataramanan R, Rosenthal JT, et al: Clin Pharmacol Ther 38:296, 1985
5. Ptachcinski RJ, Venkataramanan R, Burckart GJ: Clin Pharmacokinet 11:107, 1986
6. Venkataramanan R, Yang S, Burckart GJ, et al: Ther Drug Monit (in press)