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Abbott's Fluorescence Polarization Immunoassay for Cyclosporine and Metabolites Compared with the Sandoz "Sandimmune" RIA

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A new procedure for measuring cyclosporine in plasma has been introduced by Abbott Laboratories, involving their TDx instrumentation and fluorescence polarization immunoassay. Radioimmunoassay (RIA) and high-performance liquid chromatography are currently the conventional methods for measuring cyclosporine in plasma and whole blood. In an effort to find a method that will decrease the radioactive hazard, the reagent and supply cost, and the labor requirements associated with RIA procedures, we used specimens from transplantation patients to compare the Abbott assay with the Sandoz Sandimmune* assay. We believe that the Abbott assay offers some advantages over the Sandimmune RIA procedure, providing a reliable but simpler and less hazardous technology.

The increase in organ transplantation and in patients receiving cyclosporine as part of immunosuppressive therapy has caused a dramatic increase in the number of laboratory requests for its assay (*1*). The two technologies commonly used to evaluate cyclosporine are high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA). Abbott Laboratories has recently introduced an assay that measures cyclosporine and its metabolites by fluorescence polarization immunoassay (FPIA), with the "TDx" instrumentation (*2*).

At the University of Pittsburgh School of Medicine, 4000 patients' specimens per month are analyzed in duplicate by the Sandoz "Sandimmune" assay for cyclosporine. The method measures the drug and some fraction of metabolites in either plasma or whole blood. A competitive protein-binding ligand procedure, the Sandimmune RIA involves use of a tritiated antigen, which requires liquid scintillation spectrometry. The combination of radiolabeled materials and complex analytical techniques results in a variety of practical concerns: (*a*) radioactive hazard to personnel; (*b*) disposal of low-level radioactive waste; (*c*) expense and storage of reagents and disposables; (*d*) need for highly trained technical staff; (*e*) increased labor intensity from multiple handling steps; (*f*) lengthy incubation and counting processes; (*g*) complex and costly instrumentation, requiring significant space accommodations; and (*h*) relatively poor precision.

As the volume of cyclosporine analyses increases, the difficulties encountered in RIA procedures are magnified. We have evaluated the Abbott cyclosporine assay in order to more quickly provide results to clinicians and also to eliminate radioactive reagents from the laboratory. We analyzed 408 specimens along with more than 200 controls, in duplicate, by both the Abbott FPIA TDx method and the Sandimmune RIA. Results of this study indicate that values for cyclosporine measured by the FPIA TDx are higher than those determined by the Sandimmune RIA. The TDx method, however, is more precise, easier, and highly reliable. In addition to rapid result reporting, the method provides a simple technology with few handling steps, compact instrumentation, and few reagent storage and disposal problems.

Materials and Methods

Sample collection. Samples, collected from each patient into tubes containing EDTA as anticoagulant, were allowed to equilibrate for 2 h at room temperature, then plasma was separated from the cells at room temperature after centrifugation. Temperature of the specimen during separation is an important factor, because the equilibration of cyclosporine with erythrocytes is temperature dependent (*3*). No correction was made for hematocrit.

Procedures

RIA. Cyclosporine in plasma was measured by RIA with the Sandoz RIA kit according to the manufacturer's instructions (*4*).

FPIA. Fluorescent polarization immunoassay with use of the Abbott TDx instrument is a recognized method for measurement of drugs and other constituents in body fluids (*5–7*). In the new procedure for cyclosporine, an anti dy is used that reacts both with cyclosporine and some of its metabolites. We performed the assay according to the manufacturer's instructions.

The concentration range of the Abbott calibrators is 0 to 1000 μg/L. Specimens containing cyclosporine in concentrations >1000 μg/L were diluted with the zero calibrator (normal human serum) before the precipitation step, as were also specimens with background intensity >1000. The manufacturer does not recommend a change in the background (MX BKG) setting.

Results

Cross-reactivity and s cificity. We tested cross-reactivity with compounds listed in Table 1, whose concurrent use might interfere with the assay. Cross-reactivity was established for a specimen by adding the test compound, at a concentration of 100 mg/L, to drug-free pooled human serum, then assaying the specimen by the FPIA assay. The percentage cross-reactivity was calculated as: (measured cyclosporine and metabolites concentration divided by the concentration of the test compound) ×100. The compounds tested were all below the detection limit of the assay (<15 μg/L).

Precision. Precision studies were performed on the Abbott low-, medium-, and high-concentration control sera (Table 2). All three control concentrations were included in each batch of 20. For the low control, 59 replicates were processed, yielding a coefficient of variation (CV) of 9.5% total and 7.4% within-run; for the medium concentration, 60 replicates for a total CV of 4.9% and within-run CV of 2.9%; for the high concentration, 61 replicates with a total CV of 4.4% and within-run CV of 3.0%.

Precision of patients'-sample and control duplicates was excellent. Of about 600 specimens analyzed, only 10 samples

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Table 1. Compounds Tested for Interference in the Abbott Cyclosporine Assay[a]

Amikacin	Methylprednisolone
Ampicillin	Neomycin sulfate
Azathioprine	Oxytocin
Carbamazepine	Phenobarbital
Cephalosporin	Phenytoin
Chloramphenicol	*Prednisone
Cimetidine	*Prednisolone
Digitoxin	Primidone
Digoxin	Procainamide
Dipyridamole	Propranolol
Disopyramide	Quinidine
Encainide	Spectinomycin
Erythromycin	Streptomycin
Ethosuximide	Tobramycin
Furosemide	Tocainide
Gentamicin	Triamterene
Kanamycin A	Valproic acid
Kanamycin B	Vancomycin
Ketoconazole	Verapamil
Lidocaine	

[a] These compounds were undetectable (<15 μg/L) when tested up to 100 or *10 mg/L.

Table 2. Performance Statistics of the TDx Cyclosporine Assay

Control (Abbott)	Expected range	Determined range	Precision (n = 59 to 61) Total CV, %	Within-run CV, %
	mg/L			
Low concn	60–90	63–93	9.5	7.4
Med. concn	225–275	219–267	4.9	2.9
High concn	600–800	663–792	4.4	3.0

had to be repeated because of duplication problems (duplication criterion: samples must duplicate within 5 net Polarization Units).

Standard-curve stability. Three times during 20 days, fresh TDx standards were run in the TDx. Values consistently fell within 5% of assigned values and none differed by more than 10%.

Carryover. We saw no significant carryover from specimens with high concentrations.

Accuracy. Analytical recovery was tested with two sets of samples prepared by adding cyclosporine to give concentrations of 50, 75, 100, 200, 250, 500, and 700 μg/L to normal human serum and to normal human serum diluted fivefold in TDx Dilution Buffer. The TDx analyzer was calibrated with serum-based calibrators provided by the manufacturer. Both sets of specimens were determined in replicates of five and the results compared with the calibration curve (Table 3). The average analytical recovery from diluted serum was 98.5% (SD 3.1%), from undiluted serum, 97.2% (SD 3.8%).

Comparison of methods. We received 408 specimens with requests for cyclosporine assays. These were used in the correlation studies. Plasma samples were prepared and processed by both the Sandoz Sandimmune RIA procedure and Abbott's cyclosporine and metabolites FPIA assay. Each sample was analyzed in duplicate and the values were averaged. On linear regression analysis comparing the two sets of results, the equation for the regression line was determined to be $(1.72 \pm 0.045x) + (61.8 \pm 4.48)$ μg/L, with a correlation coefficient of $r = 0.884$ (Figure 1). The standard error of the regression was 56.4.

Table 3. Analytical Recovery of Cyclosporine in the Abbott TDx Assay

Expected concn	Recovered from diluted serum	Recovery, %	Recovered from undil. serum	
	μg/L		μg/L	%
50	50.40	100.80	49.79	99.58
75	75.15	100.20	71.39	95.19
100	96.64	96.64	96.41	96.41
200	197.53	87.77	202.98	101.49
250	257.75	103.10	254.09	101.64
500	479.50	95.90	459.79	91.96
700	660.43	94.35	657.32	93.90

Discussion

We found the Abbott TDx to be significantly more precise than the Sandimmune RIA. Coefficients of variation ranged from 4% to 10% for the TDx. Comparable values for the Sandimmune assay, which is in routine use in our laboratory, are 10% to 17%.

When our HPLC or RIA standards were run in the TDx, the regression equation resulting from that comparison was TDx value = 0.92 [HPLC or RIA] value − 3.9, $r = 0.9992$. However, values for the TDx assay from patients' samples were about 1.7 times the RIA values. This lack of correlation indicates that the two assays are not in fact measuring exactly the same thing. The Abbott antibody may react with a cyclosporine metabolite that is unreactive in the Sandoz assay system, or there may be a generally increased cross-reactivity to all or to some subset of cyclosporine metabolites. In either case we believe this offers an advantage for the TDx assay, because recent observations from this insti-

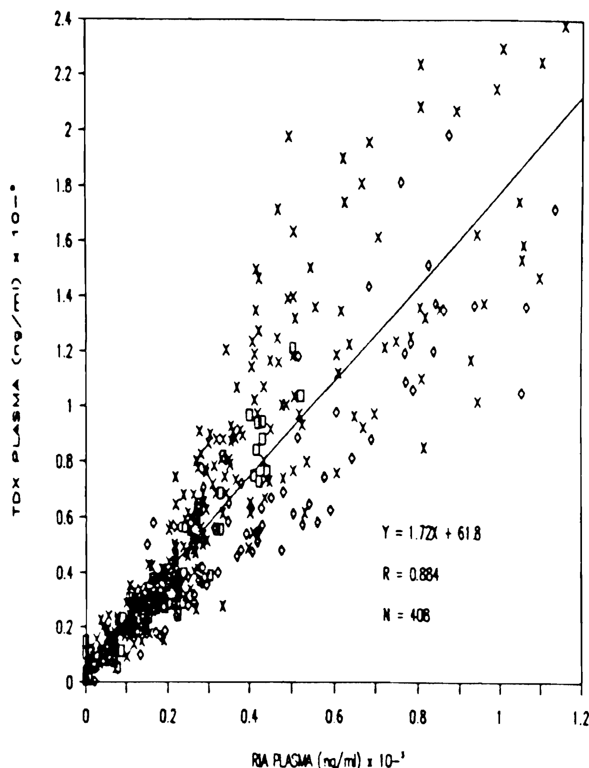

Fig. 1. Comparison of cyclosporine values obtained by the Abbott TDx and Sandoz RIA methods

Organ transplanted is as indicated: ×, liver, □, heart, ◇, kidney

tution indicate that some cyclosporine metabolites—particularly M1, M13, M17, M18, and M21—possess immunosuppressive activity as demonstrated in vitro by use of lymphocyte-proliferation assays. Metabolites M17 and M1 in particular exhibit immunosuppressive activity in vitro approaching that produced by cyclosporine. Moreover, these metabolites appear to exhibit varying immunosuppressive potency depending upon the T-cell clones used in the test system (8, 9). Therefore, the drug concentrations measured by the FPIA assay may more closely approximate the concentration of immunosuppressive drug present in the circulation. These differences between the two assay systems, coupled with the fact that there is greater imprecision with the RIA method, account for the observed correlation coefficient of 0.884 as well as the greater scatter observed at high values.

There are two potential problems with the TDx plasma cyclosporine assay: (*a*) Assay linearity of 1000 $\mu g/L$ was not sufficient in our laboratory because about four to six samples in a tray of 20 needed to be diluted. (*b*) With prolonged use, the integrity of the kit may be compromised because, over time, control values may exceed the high limit, possibly owing to some evaporation of the reagent. However, we observed no substantial evaporation during an analytical run: control values at the beginning and end of several assays were 247 ± 9.6 and 243 ± 9.4 mg/L ($n = 24$), respectively.

The TDx method offers a simpler procedure with fewer handling steps and faster turnaround time. The instrumentation is automated and compact, allowing better use of technologist time and laboratory space. Reagents and supplies present minimal storage and disposal problems and no radioactivity hazard.

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