Effects of Sample Preparation on Concentrations of Cyclosporin A Measured in Plasma

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Because cyclosporin A rapidly changes its distribution in blood with changes in temperature, sample preparation affects results for it as measured in plasma. If whole blood is stored at either 4 °C or room temperature, results for cyclosporin A in the plasma are lower than in whole blood stored at 37 °C and centrifuged at this temperature. Re-equilibration of the former to 37 °C before cells are removed increases the analytical recovery of cyclosporin A in plasma; the optimal equilibration interval is 30 min. Use of such re-equilibration, followed by immediate centrifugation at room temperature, increases values obtained for cyclosporin in plasma by 60 to 65% over those determined after non-temperature-standardized collection procedures, but does not significantly improve the correlation between values for plasma and whole blood. Hematocrit and concentrations of cyclosporin A in plasma are inversely related. Correction for hematocrit improves the correlation between results for plasma and whole blood.

Additional Keyphrases: whole blood vs plasma as samples variation, source of immunosuppressive drugs

Cyclosporin A, an immunosuppressive drug (1–3), is distributed in whole blood in the following proportions: erythrocytes 58%, lymphocytes 8 to 10%, and plasma protein 30% (4). This distribution appears to be temperature dependent, the concentrations of cyclosporin A in plasma decreasing with increasing temperature (4–8). Plasma from samples maintained at 37 °C has more than twice as much apparent concentration of cyclosporin A that maintained at 6 or 21 °C before the cells are separated (5–8). Prolonging the time between blood collection and cell removal also affects the apparent concentrations of cyclosporin A in plasma (5–7). This observation has led to the suggestion that the measurement of cyclosporin A in plasma is meaningless, and that whole blood measurements should be used instead (5).

Here we further investigate the effects on the concentration of cyclosporin A in plasma of the time and temperature of sample handling before cells are removed.

Materials and Methods

Sample Preparation

Venous blood was collected in heparinized tubes from 38 post-transplant patients receiving cyclosporin A, just before their next dose and 12 h after their last.

Non-temperature-standardized protocol. About 0.5 to 3 h after collection we set aside an aliquot of each sample for whole-blood analysis and centrifuged the remaining specimen at room temperature to remove cells. The plasma and whole-blood specimens were both then stored at −20 °C.

Temperature-standardized protocol. After collection, samples were refrigerated for no longer than 4 h, re-equilibrated to 37 °C by incubation in a water bath for 30 min, then centrifuged without delay (1100 × g, 10 min, room temperature). The resulting plasma was stored frozen at −20 °C until analysis.

Experimental Procedures

Time and temperature experiments. Aliquots of whole blood from patients receiving cyclosporin A were incubated for 0.25, 0.5, 1, 2, 4, 6, and 24 h at 4 °C, room temperature (about 22 °C), and 37 °C. At these specified times, samples stored at room temperature or 4 °C were centrifuged at room temperature; those stored at 37 °C were centrifuged at 37 °C and the plasma was decanted and frozen.

In addition, we treated aliquots of samples stored at room temperature and 4 °C for the above times by re-equilibrating to 37 °C by incubating for 15 min in a 37 °C water bath, followed by centrifugation at 37 °C and prompt removal of the plasma.

Equilibration time experiment. Aliquots of whole blood from patients receiving cyclosporin A were maintained at 4 °C for 4 h after collection, then equilibrated in a 37 °C water bath for 0, 10, 15, 30, 60, 120, and 180 min. All specimens were centrifuged at 37 °C, and the plasma was immediately decanted and stored at −20 °C until analysis.

Measurement of cyclosporin A. We measured cyclosporin A by radioimmunoassay, using tritiated cyclosporin A as tracer and an antibody in a kit supplied by Sandos Ltd., Basle, Switzerland. We used duplicate 5-mL patient’s samples, mixing them with 100 μL of tracer, 700 μL of Tris buffer (0.5 mol/L, pH 8.5), and 100 μL of sheep antisera to cyclosporin A, then incubating for 2 h at room temperature. Free and bound fractions were separated by extraction with charcoal for 12 min at 4 °C. We measured the radioactivity in each specimen with a liquid scintillation counter, using a quench-corrected counting program. The analytical recovery of cyclosporin A in plasma and whole blood supplemented with the drug was about 75%.

Correction for hematocrit. Hematocrits of samples for cyclosporin analysis were measured as part of the routine blood count. We corrected the plasma cyclosporin A results for hematocrit by multiplying the uncorrected plasma cyclosporin A results by the patient’s hematocrit/0.39, the normal value for the hematocrit.

Results

Time and temperature dependence. We investigated the time and temperature dependence of sample handling, before cells are removed, on the concentration of cyclosporin A in plasma. We treated whole-blood samples in various ways: (a) storage and centrifugation at 37 °C (suggested by the manufacturer to be the optimal procedure); (b, c) storage at 4 °C or room temperature, with centrifugation at the latter temperature; (d, e) storage at 4 °C or room temperature, followed by re-equilibration at 37 °C and centrifugation. The results (Figure 1), expressed as the mean percentage of values obtained for samples stored and centrifuged at
Son equilibration different cyclosporin to 37° C. To investigate the effect of temperature of centrifugation on recovery of cyclosporin A in plasma subsequent to re-equilibration, we re-equilibrated 16 patients' samples maintained at 4 °C to 37 °C for 30 min, followed by prompt centrifugation at 1500 x g under the following conditions: 37 °C, room temperature, and room temperature in a rotor that had been pre-warmed to 37 °C. We found no statistically significant difference (p > 0.05) in the resulting values for cyclosporin A. Evidently, after re-equilibration at 37 °C, samples can be immediately centrifuged at room temperature.

Values for plasma vs whole blood. Plasma cyclosporin concentrations correlate poorly with concentrations in whole blood, which may have been due to a lack of temperature-standardization of sample preparation before cells are removed. We measured cyclosporin A in plasma from 38 patients, using both a temperature-standardized and a non-temperature-standardized collection protocol, and correlated these values with those obtained for whole blood. Values for plasma (x) by the former and latter protocols showed similar correlation with values for whole blood (y) [(x̄ = 528, ȳ = 157, y = -16.1 + 0.33x, S_yx = 63.4), r = 0.64] vs [(x = 528, ȳ = 260, y = -18.7 + 0.52x, S_yx = 89.5, r = 0.70), respectively], while the temperature-standardized protocol on average gave mean values for cyclosporin A in plasma that were 60 to 65% higher than those by the non-temperature-standardized protocol.

Effect of hematocrit on plasma cyclosporin A concentrations. We detected many outliers in the correlation studies described above, reflected in the large standard deviation about the regression line for both the temperature-standardized (S_yx = 89.5) and non-temperature-standardized (S_yx = 63.4) collection protocols. Variations in the hematocrit may affect plasma cyclosporin A measurement, because about half of cyclosporin A in the circulation is ordinarily bound to erythrocytes (11). We found significant (p < 0.001) negative correlation between plasma cyclosporin A as determined with either the temperature-standardized (r = -0.54) or non-temperature-standardized (r = -0.63) collection protocols. When we corrected the values for cyclosporin in plasma prepared by these two protocols for hematocrit as described above, we found an improved correlation and decreased scatter about the regression line for the correlation between cyclosporin A concentrations in plasma (x) and whole blood (y) for both the former (x̄ = 528, ȳ = 141, y = 13.5 + 0.24x, S_yx = 40.8, r = 0.70) and the latter (x̄ = 528, ȳ = 235, y = 23.1 + 0.40x, S_yx = 55.7, r = 0.77) protocols.

Discussion

We have confirmed the observations of others regarding the temperature dependence of values obtained for cyclosporin A in plasma (4–9). Both our results and the results of others (9) have shown poor correlation between plasma and whole-blood concentrations of cyclosporin A even with a temperature-standardized collection protocol. Given the nature of the distribution of cyclosporin A in whole blood, correction for variations in hematocrit improved the correlation. Because the drug avidly binds to lipoproteins and leukocytes (4), correction for variations in these analytes may also improve the correlation. The measurement of cyclosporin A in whole blood has been recommended (5) to alleviate the problems encountered in sample handling and drug distribution described here. Further work in this area is necessary.

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Combined Immunochemical and Electrophoretic Determinations of Proteins in Paired Serum and Cerebrospinal Fluid Samples

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Albumin and immunoglobulin G were simultaneously determined in paired serum and cerebrospinal fluid samples from healthy volunteers. The albumin quotient and the IgG index were calculated to establish normal values. High-resolution electrophoresis was also performed with the same samples and the electrophoretic patterns were correlated with the numerical values to improve the reliability of the test results. Representative examples from abnormal conditions are also shown, to illustrate the increased accuracy and useful applications of this combined analysis in the diagnosis of neurological conditions.

Additional Keyphrases: reference interval · nervous-system disorders

Determination of proteins in paired samples of serum and cerebrospinal fluid (CSF) is performed in the clinical laboratory to demonstrate two common pathological conditions: (a) blood–brain barrier impairment, because increased concentrations of serum components in the CSF may have a damaging effect in the central nervous system; and (b) a selective increase in immunoglobulin G (IgG) in CSF produced locally, because this is indicative of immunological activity in the central nervous system.

The recent development of immunochemical techniques for simultaneously determining albumin and IgG in serum and CSF, followed by estimation of the albumin quotient and IgG index, stimulated the study of patients with neurological disorders (1–3).

In the present study, albumin and IgG concentrations were determined in paired serum and CSF samples from healthy subjects, to establish normal values for the albumin quotient and the IgG index. "High-resolution" zone electrophoresis also was performed in the same samples, to serve as a quality control of the numerical values and to demonstrate oligoclonal banding. Selected examples of increased permeability and abnormal electrophoretic patterns also are presented, to show the potential benefit of the combined immunochemical analysis and high-resolution electrophoresis of serum and CSF for evaluating patients with neurological disorders.

Materials and Methods

Paired CSF and blood samples were obtained by routine procedures from 20 healthy volunteers (16 men, four women; ages 20–87 years) after informed consent. Their health status was established by rigorous medical and neurological examinations and laboratory screening. Laboratory screening included chemical (SMAC analyzer) and hematological tests. Individuals were excluded from the study if there was any evidence of cardiovascular, cerebrovascular, and neurosensory disorders, or if they had any history of drug or alcohol abuse or a major psychiatric disorder. The samples were either analyzed on the same day that they were obtained or after refrigeration overnight at 4°C. CSF (2.5 mL) was concentrated 80-fold in a Minicon Concentrator CS-15 (Amicon Corp., Danvers, MA). Five microliters of the concentrated CSF and 1.5 μL of a paired serum sample were used for electrophoresis on microscope slides covered with agarose gel. Both samples were run simultaneously. After electrophoresis for 13 min, the proteins were fixed and stained with Amido Black as previously described (4, 5).

Albumin and IgG in paired serum and CSF samples were determined simultaneously by radial immunodiffusion on commercially supplied plates (Kallestad Corp., Austin, TX). Three standards and a control were run with each set of unknowns. The albumin quotient is the ratio of the CSF concentration to the serum concentration of albumin multiplied by 10⁴. The CSF IgG index was calculated by using the formula:

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\text{IgG index} = \frac{(\text{CSF IgG/serum IgG})}{(\text{CSF albumin/serum albumin})}
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