Citation Classic

Liquid-Chromatographic Determination of Cyclosporin A in Blood and Plasma

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Featured Article: Sawchuk RJ, Cartier LL. Liquid-chromatographic determination of cyclosporin A in blood and plasma. Clin Chem 1981;27:1368–71.[2]

Shortly after the introduction of cyclosporin A in the late 1970s, much controversy surrounded the notion of a therapeutic range for this drug. Although evidence suggested a nephrotoxic range (1, 2), clinicians had little guidance as to what concentrations in blood or plasma should be maintained to ensure adequate immunosuppression. Indeed, questions surrounded the choice of plasma vs whole blood as the preferred matrix for monitoring patient response. There clearly was a need for a reliable, specific, and relatively rapid assay for cyclosporin A in biological matrices. Linda Cartier and I published this paper to describe such a method for the quantitfication of this drug in the blood and plasma of transplant patients.

One of the factors contributing to the difficulty in identifying a therapeutic range for cyclosporin A was the large amount of variability observed in plasma concentrations of this peptide in a given patient receiving the same dose over an extended period of time. This intrapatient variability was explained by unpredictability in the absorption of the drug. Eventually, absorption variability was attributed in part to a significant "first-pass" metabolic effect related to hepatic and intestinal metabolism as the drug was absorbed into the systemic circulation. An additional factor was the limited and variable absorption of the drug from the dosage form available at that time. Circadian and food effects were also likely involved.

In addition to these factors, however, another source of uncertainty appeared to exist in the routine monitoring of transplant patients receiving cyclosporin A. Because of the inherent properties of this cyclic peptide, its distribution between plasma and erythrocytes was dependent on the patient's hematocrit, the concentration of the drug in the blood sample,

and how long the sample sat on the bench at room temperature before centrifugation. Recognition of this ex vivo redistribution of this immunosuppressive agent between the components of whole blood led to the idea that the drug concentration might be more reliably measured in whole blood, because this metric was not subject to the postsampling artifacts that contributed to the variability seen in the measurement of plasma concentrations.

Transplant surgeons and clinicians who were treating patients with immunosuppressive therapy believed that cyclosporin exhibited a narrow therapeutic index and were aware of the difficulties in interpreting plasma concentrations. These healthcare professionals therefore sought a reliable analytic method for quantifying cyclosporin in whole blood. Responding to a request from our clinical colleagues at the University of Minnesota, we developed an HPLC method for the determination of whole blood concentrations of cyclosporin A.

The analytical method we established involved solvent extraction with a backwash to remove late-eluting endogenous compounds. Although chromatographic peaks of this peptide on a standard C18 column were broad and tailed unacceptably at room temperature, we found that peak shape and symmetry could be optimized, even without gradient elution, at a column temperature of 75 °C. In spite of the modest absorptivity of the peptide, the sample-extraction and clean-up procedures provided adequate peak signals by monitoring at a low ultraviolet wavelength. A previously reported method for the assay of this drug in plasma and urine had clearly established that an increased column temperature was needed to optimize peak shape. This method, however, required gradient elution and chromatographic times of about 30 min (3). The method we developed allowed for run times of about 10 min. Because this assay was relatively rapid, and the limit of quantification for cyclosporin A was 25 μg/L with 2 mL of whole blood, it was adopted by numerous clinical laboratories participating in the monitoring of transplant patients.

Many clinicians now believe that a meaningful measure of a patient's exposure to cyclosporin A may be obtained by measuring drug concentrations in the patient's blood over the first few hours of a dosing

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interval and integrating these values to calculate a partial area under the curve (4). It has been suggested that the analytical burden may be substantially reduced if such area under the curve calculations are obtained by assaying a single sample consisting of pooled individual serial samples obtained over time (5). Additional insight into the therapeutic effects of cyclosporin A will likely be gained by monitoring cyclosporin A blood concentrations, identifying appropriate biomarkers, and developing mechanism-based pharmacodynamic models for assessing immunosuppression and clinical outcome.

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Editor's and Author's Comments:
This figure, taken from the highlighted paper, shows the chromatography for the method by Sawchuk and Cartier. The left chromatogram is for a blank blood and the right chromatogram for a patient blood containing 152 ng/mL cyclosporin A (peak 2) and the internal standard (peak 3). Although numerous clinical laboratories were able to take advantage of the selectivity and reliability that HPLC-ultraviolet offered for the quantification of cyclosporin A, this benefit was offset by the large specimen volume required and the multistep sample preparation and cleanup required prior to analysis. Sample preparation involved (*a*) extraction of 2.0 mL blood with ether, (*b*) transfer and evaporation of the organic phase, (*c*) reconstitution and cleanup under acidic conditions with hexane, (*d*) removal and discard of the hexane, (*e*) a second extraction of the blood under alkaline conditions with ether, (*f*) transfer and evaporation, and finally (*g*) reconstitution in mobile phase and chromatographic analysis. With introduction of newer detection methods such as tandem mass spectrometry, specimen volume requirements have decreased to less than 50 μL, and sample preparation has been reduced to a 1-step protein precipitation.