Cyclosporin Concentrations in Whole Blood and Plasma

To the Editor:

Debate continues on the usefulness of measuring cyclosporin in plasma as a potential indicator of its immunosuppressive or toxic effects (1–3). In September 1982, the manufacturer (Sandoz Products Ltd., Basle, Switzerland) circulated to users details of the apparent variation in affinity of the drug for erythrocytes at various temperatures, work that has since been confirmed (4). It has been suggested that a rigorous routine of incubating the blood sample before the plasma is separated is necessary to standardize results, and an incubation temperature of 37°C has been recommended (5).

We measured cyclosporin in both whole blood and plasma after a 30-min incubation at 37°C in a single patient who was undergoing therapy with cyclosporin during bone-marrow transplantation. We used the radioimmunoassay method provided by Sandoz Products Ltd. When we compared the two sets of data, we found a poor correlation (y = 0.26x – 62.6, r = 0.58, n = 34, where y = values for plasma and x = values for whole blood), although we strictly controlled sample preparation. The whole blood/plasma concentration ratio varied from 1.6 to 3.6.

These observations would appear to support suggestions that the degree of uptake of the drug by erythrocytes depends on the total drug concentration and on binding to other blood components, including lipoproteins (6). Whatever the cause, the variations are such that even strict adherence to a standardized sample-handling procedure is not sufficient to validate the use of data on concentrations in plasma from one laboratory to another—or even within the same laboratory. If figures for whole blood are used, it seems likely that the variations in sample handling, both within and outside the laboratory and among patients, will be minimized. A more widespread use of whole blood as the sample may expedite the development of a useful therapeutic range of this increasingly important drug.

References

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Comparison of Two Colorimetric Assays for Angiotensin-Converting Enzyme Activity

To the Editor:

Although angiotensin-converting enzyme (ACE; peptidyl-Dipeptidase hydrolyase, EC 3.4.15.1) plays a crucial role in blood-pressure regulation (1), its measurement in serum has also proved useful in the diagnosis and management of sarcoidosis (2, 3), the activity being increased in most patients with the active disease and declining with spontaneous remission or steroid therapy.

We previously reported a sensitive, precise assay for serum ACE, in which hippurate released from hippuryl-l-histidyl-l-leucine by ACE is quantified by reaction with cyanuric chloride/dioxan reagent in the presence of phosphate buffer (4). Notwithstanding a large unexplained constant error (y-intercept = 10.5 U/L), results by our method have been shown (5) to correlate well (r = 0.988) with those by a radiometric assay.

Recently, we had the opportunity to compare our method with the colorimetric technique of Kasahara and Ashihara (6). In this technique p-hydroxyphenylipiric acid, released from p-hydroxyhippuryl-l-histidyl-l-leucine by ACE, is hydrolyzed by hippuricase (EC 3.5.1.14) to p-hydroxybenzoic acid and glycine. Oxidative coupling of p-hydroxybenzoic acid with 4-aminoantipyrine produces a quinoneimine dye. ACE activity is calculated from absorbance measurements at 505 nm. This method is now available in kit form ("ACE-color"; Fujizoki Pharmaceutical Co. Ltd., Shinjuku-ku, Tokyo, Japan; New Zealand agent, Medic DDS Ltd., Wellington, N.Z.). A gracious gift of some kits by the manufacturer enabled us to compare the measurement of serum ACE by these two methods.

We assayed 40 sera with a wide range of ACE activities by both methods. Linear regression analysis of the data for the kit method (y) vs our method (x) gave the equation y = 0.273x – 0.436 (r = 0.993, S_yx = 0.973, x = 67.73, SDx = 30.54, y = 18.08, SD_y = 8.40). A high degree of correlation, with minimal constant error, is evident. Although the chemical sensitivity of the kit method is about one quarter that of our method (alope = 0.273), the precision of the kit is excellent. Replicate analyses (n = 10) of two sera resulted in CVs of 2.2% at 7.8 U/L and 1.8% at 14.6 U/L. These CVs compare favorably with data of Kasahara and Ashihara (6) and with CVs for our method. Reference intervals were not quoted by Kasahara and Ashihara, but application of the regression equation to our figures gave reference intervals for adults of 6–22 U/L (men) and 6–18 U/L (women).

The ACE-color test kit should be welcomed by those clinical laboratorians who wish to assay serum ACE activity but have hitherto been hampered by the lack of a kit method.

References

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Improved Simultaneous Liquid-Chromatographic Determination of Antiepileptic Drugs in Plasma

To the Editor:

"High-performance" liquid chromatography (HPLC) is currently commonly used in monitoring antiepileptic