


42. Mendel CM, Cavaleri RR. Inability to detect an inhibitor of T4-serum protein binding in sera from patients with nonthyroidal illness [Abstract]. 64th meeting, Am Thyroid Assoc 1989:T-5.

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**Therapeutic Monitoring of Cyclosporine: Impact of a Change in Standards on 125I-Monoclonal RIA Performance in Comparison with Liquid Chromatography**

Paul A. Keown,1,2 Jane Glenn,1 Jorge Denegri,1 Ursula Maciejewska,1 David Seccombe,1 Marilyn Stawicki,4 David Freeman,4,5 Calvin Stiller,4 Christopher Shackleton,5 Eugene Cameron,2 and G. Phillips2

This study examines the measurement of cyclosporine (CsA) by 125I-monoclonal RIA, and describes the impact of the recent change in the standard curve provided. CsA concentrations in serum and whole-blood control samples measured by 125I-RIA were initially 6–18% higher than those by HPLC. During the first two months of 1989, a significant and sustained deviation in the 125I-RIA produced results that exceeded the HPLC results by 21–28% (P < 0.001). Introduction of the new standard curve in March 1989 returned the concentration of the whole-blood controls to the previous range (11–12% above HPLC, P < 0.001). Measurement of clinical samples from heart, liver, and bone-marrow graft recipients by 125I-RIA by both old and new kit standards produced a close linear correlation (y = 0.89 x – 19.02; r = 0.99; n = 75, range = 40–850 μg/L), with use of the new standards yielding results 82% of SD (8%) of those with the preceding assay. However, even with the new standard curve, CsA concentrations by 125I-RIA in the clinical samples exceeded those by HPLC by a factor of 1.37 (SD 0.18) to 1.52 (SD 0.19). Segregation for transplant type did not affect the RIA/HPLC ratio. The results suggest cross-reactivity of the 125I-RIA with material present in vivo.

Additional Keyphrases: control materials · variation, source of

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Materials and Methods

Control samples: We used four separate control samples: two serum controls prepared by INCStar Ltd., provided with the assay, and an assay and whole-blood controls prepared at the participating centers, i.e., fresh, pooled whole-blood collected in EDTA. This whole blood was frozen, thawed, and diluted with an equal volume of Tris buffer (50 mmol/L, pH 8.5), then mixed with CsA added from a 40 mg/L stock solution (Sandoz Ltd.) to produce final CsA concentrations of 200 and 500 μg/L.

Clinical samples: Whole-blood samples in EDTA were obtained from 75 patients at 1 day to 156 months after renal (n = 56), bone marrow (n = 13), or heart (n = 6) transplantation at the Vancouver General Hospital (VGH). Kidney and heart-graft recipients received induction antithymocyte globulin or prophylactic immunosuppression with OKT3 antibody, followed by triple maintenance therapy (prednisone, azathioprine, and CsA); bone-marrow recipients were treated with CsA and methotrexate. Samples for CsA measurement were taken 10–12 h after the previous dose, and were either measured within 4 h or stored at −70 °C for subsequent analysis. We have previously found that the concentration of CsA is unaltered by storage for up to six months in this manner (data not presented).

RIA measurement: All samples were analyzed by RIA at VGH, the INCStar-SP kit being used as recommended by the manufacturer. The range of the standard curve was 3–1560 μg/L, and CsA concentration was calculated by using the spline function. The sensitivity (detection limit) of the assay was approximately 25 μg/L, with a lower clinical limit of 40 μg/L. Above this threshold, the mean interassay CV was <10%. The standard deviation index (SDI) of this laboratory in the Canadian Quality Assurance Programme (n = 28 centers), calculated from the formula SDI = laboratory result − mean result ÷ SD of the mean, was 0.03–0.24. The SDI indicates the position of the value reported by a specific laboratory in the distribution of all such values reported. A value <1.50 is considered to be acceptable.

HPLC measurement: To minimize the bias implicit in analysis within a single laboratory, all HPLC analyses were performed independently in two Canadian centers. Analysis at VGH involved use of an HP 1090 liquid chromatograph (Hewlett-Packard, Ft. Collins, CO) and a 15 cm × 0.46 cm Maxsil (Phenomenex, Torrance, CA), 5-μm particle size C18 column maintained at 70 °C. The mobile phase consisted of acetonitrile/water (73/27 by vol) at a flow rate of 1 mL/min. Before injection, samples were extracted by using diethyl ether and an LC-Si (Supelco, Oakville, Ont., Canada) minicolumn.

University Hospital, London (UHL), used a modification of the technique of Carruthers et al. (8), with a silica solid-phase extraction procedure (9). They used a Waters HPLC system (Waters, Milford, MA) with a 15 cm × 0.32 cm Spherisorb (Phase Sep, Norwalk, CT) 5-μm particle size C8 column maintained at 100 °C. The mobile phase, acetonitrile/methanol/water (47/27/33 by vol), was run at a flow rate of 0.8 mL/min.

In both methods the effluent was measured at 210 nm, and CsA was quantified by using internal standardization and peak areas. Linearity was established for both assays between 40 and 700 μg/L, and within- and between-assay CVs were 6–10%. Whole-blood or serum-based standards were used for measurement of whole-blood and serum specimens, respectively.

Results

The characteristics and CsA concentrations in the four control samples are shown in Table 1. During the last third of 1988, the concentrations determined by the CYCLO-TRAC-Sp (RIAperiod 1) were 8–18% higher than those by HPLC in both serum and whole-blood controls. The measurements remained consistent throughout this period of study.

There was then a significant and unexplained increase in assay results over the next two months (RIAperiod 2), during which the CsA concentrations of the whole-blood controls were 21–28% greater than the HPLC values. There was

| Table 1. Change in CsA Concentration (μg/L) of Control Samples Measured by the 125I-Monoclonal RIA |
|--------------------------------------------------|----------|----------|----------|----------|
| 4PCLa                                           | Kit lot  | Serum 1  | Serum 2  | Blood 1  |
| RIAb                                           | n/a      | 98 ± 1   | 318 ± 8  | 197 ± 11  |
| Period 1                                       |          |          |          |          |
| Sept 1988                                      | 340168   | 114 ± 12c | 348 ± 25c | 229 ± 21  |
| Oct 1988                                       | 390318   | 116 ± 9c  | 348 ± 33c | 218 ± 20  |
| Nov 1988                                       | 430088   | 104 ± 11c | 349 ± 34c | 214 ± 15  |
| Dec 1988                                       | 640088   | 111 ± 15c | 342 ± 36c | 224 ± 19  |
| Period 2                                       |          |          |          |          |
| Jan 1989                                       | 510348   | 143 ± 13c | 477 ± 45h | 242 ± 19  |
| Feb 1989                                       | 050219   | 147 ± 14e | 460 ± 44f | 251 ± 25  |
| Period 3                                       |          |          |          |          |
| Mar 1989                                       | 090089   | 125 ± 13m | 404 ± 27e | 213 ± 17m |
| Apr 1989                                       | 110159   | 123 ± 20e | 417 ± 35g | 225 ± 22  |

* Mean ± SD of 4–20 measurements.
| Mean ± SD of 22–54 measurements.

Significantly different from Dec 1988 result (P <0.001).

Significantly different from Feb 1989 result (P <0.001).

n/a: not applicable.
also a marked increase of CsA in the two serum controls supplied, although the manufacturer had indicated an adjustment in the expected results. This deviation was not due to a deterioration in the whole-blood controls or a decrease in the precision of the assay. Concordance between sequential control lots was excellent when determined by HPLC: Control 3, period 1, 198 ± 8 μg/L, n = 9; period 2, 201 ± 11 μg/L, n = 9; Control 4, period 1, 496 ± 21 μg/L, n = 10; period 2, 501 ± 16 μg/L, n = 10. The CV of the assay showed no significant trend with time at either concentration (period 1, 9%; period 2, 9%).

With the introduction of the new standard curve in March 1989 (RIA₁₆, period 3), the CsA concentration of the whole-blood controls decreased to the previous range of 11–12% above HPLC values. The CsA concentrations of the serum controls provided with the RIA kit also fell but remained at least 25% higher than previous values, possibly reflecting a change in the control material provided.

Measurement of the 75 clinical samples (range 40–850 μg/L) by RIA, with use of both old and new serum standards from the RIA kit, produced a close linear correlation (RIA₁₆, period 3 = 0.89 RIA₁₆, period 2 - 19.02, r = 0.99). CsA concentrations obtained with the new standards (RIA₁₆, period 3) were a mean of 82 (SD 8%) of those measured with the standards from the preceding two months (RIA₁₆, period 2). There was no significant difference in this correlation between the three populations of kidney, heart, and bone-marrow recipients tested.

There was close agreement between the HPLC measurements of the same samples by HPLC in the two centers (VGH = 0.89 UHL + 1.51; r = 0.98). The correlation between RIA₁₆, period 3 with the new standards and HPLC in these centers is shown in Figure 1. Despite the introduction of the new standard curve, direct mathematical comparison showed that concentrations of CsA measured by the ¹²⁵I-RIA exceeded those determined by HPLC by a factor of 1.37 (SD 0.18) in VGH, and 1.52 (SD 0.19) at UHL throughout the range examined. There was no clear impact of the type of transplant on this relationship.

**Discussion**

Techniques that selectively detect the parent CsA molecule have been recommended as most appropriate for therapeutic monitoring of this drug (10). Despite the use of a monoclonal antibody specific for the parent drug, the results reported here suggest that the concentrations of CsA determined by the specific ¹²⁵I-RIA are not identical to those measured by HPLC. This discrepancy appears to be partly due to the standards provided with the ¹²⁵I-RIA kit. The constant error evident in the first period of use was accentuated by an acute and unexplained drift in accuracy during the months of January and February 1989. During this period the disparity between the concentrations of CsA measured by ¹²⁵I-RIA and HPLC in whole-blood controls doubled from 8–18% to 23–28%. After the introduction of new standards recalibrated to USP CsA, the accuracy of the ¹²⁵I-RIA improved to previous values but did not produce results identical to those by HPLC. This remaining difference has been attributed to inappropriate assignment of standard values (2) or a difference in the matrix composition of these standards (4), and have prompted some centers to introduce an internal serum or whole-blood standard curve in place of the serum standards provided.

The discrepancy between the ¹²⁵I-RIA and HPLC was even more marked with the clinical samples tested, suggesting that CsA metabolites or other components present in the blood of the graft recipient may contribute to this error. With few exceptions (11,12), these results are consistent with the findings reported from both single-center and multicenter quality-assurance programs, which indicate that CsA concentrations by ¹²⁵I-RIA exceed those by HPLC by 20–40% (2–6). In vitro analysis of the primary monoclonal antibody by Quesniaux et al. (1) demonstrated only limited cross-reactivity with the major CsA metabolites modified at positions 1, 4, 6, and 9. This specificity is largely conserved within the ⁹H-RIA (Sandimmun SP), for which the RIA:HPLC ratio for clinical samples reportedly ranges from 0.96 to 1.20 (2,13,14), and is superior to that obtained with the ¹²⁵I-RIA utilizing the same antibody. Possibly, technical modifications incorporated in the latter assay may alter the specificity or binding characteristics of the antibody such that primary or secondary metabolites are more easily recognized.

Despite this disparity, the ¹²⁵I-RIA remains an important assay for clinical monitoring and bears a consistent proportionality to HPLC even under the stringent condi-

![Fig. 1. Relationship between CsA concentrations in clinical samples measured by ¹²⁵I-RIA and HPLC at the two participating centers](image-url)
tions of liver and heart transplantation (2,3). The therapeutic range adopted with this assay cannot be assumed to be identical to that established by HPLC, however, and must be defined on the basis of the operating characteristics of the assay within a given center, preferably in a prospective study. The data presented here suggest that, if such a range was established before January 1989, it should not be adjusted with the introduction of the new standards.

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References

[Abstract]

Benzalkonium Interference with Test Methods for Potassium and Sodium

Thomas R. Koch and Janine Denis Cook

Six automated instruments that measure sodium and potassium were tested for interference from two compounds used in catheters. Tridodecylmethylammonium heparin did not interfere with any of the methods. However, benzalkonium heparin falsely increased sodium measurement with the Kodak Ektachem, and falsely increased potassium measurements with three instruments (Beckman Astra, Baxter Paramax, and the Instrumentation Laboratory Monarch) in which on-selective electrodes measure potassium in diluted serum. Three instruments in which ion-selective electrodes measure serum directly—Du Pont Dimension, Abbott Spectrum, and Kodak Ektachem—experienced no interference with potassium measurements. Interference of benzalkonium with potassium measurements may result from its interaction with the electrode membranes, which is accentuated in diluted serum.

Abnormalities of potassium and sodium are among the serious life-threatening disorders encountered frequently in acutely ill patients that are correctable by appropriate fluid therapy. Consequently, erroneous measurements of serum potassium or sodium have the potential to cause therapeutic errors that could result in serious injury or death to patients.

Previous reports (1, 2) have described the interference of benzalkonium (used in combination with heparin as a coating material for catheters) with the Kodak Ektachem test for sodium and with the Beckman Astra test for potassium. Here, we report results of experiments designed to study possible effects of benzalkonium with other methods for sodium and potassium. Also, we investigated for possible interference an alternative compound in catheters used by one manufacturer.

Materials and Methods


Analytical systems: Astra-6 (Beckman Instruments, Inc., Brea, CA 92621); Ektachem 700 (Eastman Kodak Co., Rochester, NY 14650); Paramax (Baxter Healthcare Corp., Irvine, CA 92718); Parallel (American Monitor Co., Indianapolis, IN 46268), flame-photometric reference test