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Analytical Performance of Microparticle Enzyme Immunoassay and HPLC-Tandem Mass Spectrometry in the Determination of Sirolimus in Whole Blood, Paul Salim, Paul J. Taylor, and Peter 1. Pillans (* Department of Medicine, University of Queensland and 2 Department of Clinical Pharmacology, First Floor Lions Clinical Research Bldg., Princess Alexandra Hospital, Ipswich Rd., Brisbane, Queensland, Australia 4102; * author for correspondence: fax 61-7-3240-5031, e-mail Psalm@medicine.pa.uq.edu.au)

The potent immunosuppressant agent sirolimus (rapamycin; Wyeth-Ayerst) is undergoing clinical trials in solid-organ transplantation (1, 2). Evidence suggests that therapeutic drug monitoring of trough sirolimus concentrations may be beneficial for two fundamental reasons: (a) a strong correlation between the trough concentration and the area under the concentration-time curve has been shown; and (b) a strong correlation between the evidence of toxicity and trough concentrations substantially >15 µg/L has been reported (3). To date, there have been only HPLC methods available to measure sirolimus in human whole blood. These include HPLC with mass spectrometric detection (4, 5) and HPLC with ultraviolet detection (6–9). As an alternative, a semiautomated method utilizing the IMx analyzer (Abbott Diagnostics) that incorporates microparticle enzyme immunoassay (MEIA) technology has been developed. There are two versions of the MEIA for sirolimus in whole blood, the prototype version (Mode 1A) and the recently manufactured premarket version (Mode 1C). Both of these assays are undergoing evaluation based on their analytical performance and clinical use.

This study compares the analytical performance of the prototype MEIA (Mode 1A) with HPLC-tandem mass spectrometry (HPLC-MS) within one center through evaluation of interference with endogenous and exogenous compounds, imprecision, recovery, and the quantification range. In addition, blood samples from renal transplant patients receiving sirolimus therapy are compared using both methods. Throughout this study, HPLC-MS was performed as per our reported method (4) and MEIA according to manufacturer’s instructions. All patient specimens were stored at −75 °C until analysis.

Investigation into potential interferences in HPLC-MS and MEIA in relation to endogenous compounds was determined based on 209 samples collected into EDTA tubes from 23 renal transplant recipients not receiving sirolimus therapy. In patient samples with no sirolimus detected by HPLC-MS (<0.2 µg/L), MEIA recorded a mean sirolimus concentration of 0.2 µg/L, where the measured concentrations were 0.0–2.5 µg/L. Potential interference from some exogenous compounds was investigated. Tacrolimus (60 µg/L), cyclosporin A (1000 µg/L), and mycophenolic acid (50 mg/L) were supplemented into calibrator A (drug-free whole blood supplied by the manufacturer). No interference was detected by HPLC-MS (<0.2 µg/L) or by MEIA (<1.3 µg/L). The recorded MEIA values were below the mean sensitivity of the assay reported by Blonski et al. (10) of 1.4 µg/L.
The inter- and intrabatch imprecision (expressed as the CV) and recovery (expressed as a percentage of the weighed-in concentration) for HPLC-MS and MEIA were investigated. The intrabatch imprecision (11%) and recovery (95.2–103%) by HPLC-MS for concentrations within the range 0.2–50.0 µg/L have been reported previously (4). The intrabatch imprecision and recovery by MEIA at three quality-control concentrations of 5.0, 11.0, and 22.0 µg/L (n = 5) were 5% and 98.8–109%, respectively. The interbatch performance of HPLC-MS and MEIA was determined through the course of clinical studies and comprised 55 and 174 batches, respectively. HPLC-MS was assessed at 0.5, 20.0, and 75.0 µg/L, yielding an interbatch imprecision, 10% and a recovery of 98.8–101%. MEIA revealed similar analytical performance at 5.0, 11.0, and 22.0 µg/L, with an interbatch imprecision <12% and a recovery of 94.2–103%. These data are summarized in Table 1.

Because HPLC-MS has a wider analytical range (0.2–100.0 µg/L) than MEIA (0.0–30.0 µg/L) and sirolimus concentrations >30.0 µg/L can be expected in some patient specimens, a higher limit of quantification outside the MEIA calibration range was investigated. This limit was determined using an in-house control prepared at 150 µg/L, which was diluted 1:10 using calibrator A and assayed in replicate (n = 5). Analysis by MEIA at 150 µg/L yielded an interbatch imprecision of 5.3% and a recovery of 93.2%. The lower limit of quantification was determined based on the criteria of Shah et al. (11), where an imprecision of less than 20% and recoveries of 80–120% for the lowest concentration are deemed acceptable. To identify the lower limit of quantification, the analytical performance at 1.5 µg/L (n = 5) was investigated, giving rise to an interbatch imprecision of 19% and a recovery of 83%. However, interferences from endogenous compounds can exceed 1.5 µg/L, suggesting that the acceptable lower limit of quantification by MEIA that can discriminate between sirolimus and interfering endogenous compounds is between 2.3 and 5.0 µg/L (the lowest control). To confirm this assumption, the lowest non-zero calibrator (3.0 µg/L) was analyzed in replicate (n = 5) by MEIA. This yielded an acceptable interbatch imprecision (4.9%) and recovery (96.7%).

The highest recorded MEIA interference based on this study was 2.3 µg/L, which is consistent with the maximum concentration detected from drug-free specimens by

<table>
<thead>
<tr>
<th>Method</th>
<th>Sirolimus,* µg/L</th>
<th>Mean ± SD, µg/L</th>
<th>CV, %</th>
<th>Recovery, b %</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC-MS</td>
<td>0.5 0.50 ± 0.05</td>
<td>9.5 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.0 19.7 ± 1.3</td>
<td>6.8 98.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>75.0 75.6 ± 4.2</td>
<td>5.5 101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEIA</td>
<td>5.0 4.7 ± 0.5</td>
<td>11 94.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.0 10.9 ± 1.2</td>
<td>11 98.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.0 22.7 ± 1.9</td>
<td>8.4 103</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Expected sirolimus concentration in the weighed-in quality control sample.

b Recovery was determined as the mean concentration of the control divided by the weighed-in concentration and expressed as a percentage.

Fig. 1. Sirolimus blood concentrations, measured by MEIA and HPLC-MS (A), and difference between the MEIA and HPLC-MS sirolimus concentrations compared with the mean sirolimus concentrations (B) in samples from 25 renal transplant recipients.

(A), n = 125. The solid line represents the line of identity (i.e., slope = 1). (B), n = 125. The solid line represents the line of best fit, and the dashed lines represent the 95% confidence intervals for the differences.
The sirolimus concentrations from a total of 125 blood samples obtained from 25 renal transplant patients receiving sirolimus therapy were measured by both methods and are shown in Fig. 1A. The range of whole blood sirolimus concentrations measured by HPLC-MS was 2.1–25.6 μg/L; by MEIA the range was 3.2–41.6 μg/L. Regression analysis of the data yielded the equation for the line of best fit as: MEIA = 1.39 (± 0.04) × HPLC-MS + 1.30 (± 0.46) μg/L (r = 0.951; S_x/y = 2.46; n = 125). These data were further analyzed by the method described by Bland and Altman (13). A plot of the difference in sirolimus concentrations (MEIA minus HPLC-MS) against the mean sirolimus concentration as measured by both methods is shown in Fig. 1B. The line of best fit and 95% confidence intervals have been shown. The equation for the line of best fit is: MEIA - HPLC-MS = 0.38 (± 0.03) × mean concentration + 0.36 (± 0.39) μg/L (r = 0.779; S_x/y = 2.02). Based on the 95% confidence intervals, it can be determined that at mean concentrations of 10 and 30 μg/L, MEIA will give corresponding estimates of 10.1–18.6 and 37.8–46.2 μg/L, respectively. The mean bias expressed as a percentage ± the SD between the two methods for renal transplant patient samples was 41.9% ± 15.3%.

Unlike HPLC-mass spectrometers, the IMx analyzer is a common instrument in clinical laboratories and is less labor intensive, with shorter sample preparation and analysis time. Thus, if the analytical performance in relation to sirolimus patient samples was similar between the methods, then MEIA could be used as an alternative to HPLC-MS. The acceptable imprecision and recovery data for HPLC-MS for the three quality controls confirm our previous validation (4). Similarly, MEIA had acceptable quality control imprecision and recovery data, which is consistent with reported MEIA studies that were based on a smaller number of batches (10, 12). The positive mean bias of 41.9% is potentially attributable to the cross-reactivity of the MEIA antibody with circulating sirolimus metabolites. The overestimation we observed was greater than that cited by Holt et al. (12), who reported a mean bias of 19.8%. The differences in the observed overestimation between these studies may be attributable to variations in the metabolite patterns in the renal transplant groups investigated.

We conclude that both HPLC-MS and MEIA (Mode 1A) have suitable analytical performance, based on weighed-in quality control samples, within the clinically relevant range (14). However, as with other immunoassays used for quantification of immunosuppressant drugs (15, 16), the degree and clinical importance of the observed overestimation in patient samples is yet to be determined. An understanding of this potential problem may be clarified through longitudinal studies from large renal transplant populations.

References

Enzymatic Assay of Calcium in Serum with Phospholipase D, Yoshitaka Morishita,1 Yoshitsugu Inuma,2 Nobuo Nakashima,1 Akira Kadota,2 Akira Miki,2 and Toshio Tadano2
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Various methods for determining calcium in body fluids have been reported. Atomic absorption spectrophotome-