Evaluation of the tacrolimus II microparticle enzyme immunoassay (MEIA II) in liver and renal transplant recipients

JAN L. COGILL,[1] PAUL J. TAYLOR,[1*] IAN S. WESTLEY,[2] RAYMOND G. MORRIS,[2]
STEPHEN V. LYNCH,[3] and ANTHONY G. JOHNSON[1]

We evaluated the MEIA II with blood samples with added tacrolimus (3.0, 5.0, 11.0, and 22.0 μg/L). The assay had acceptable recoveries (99–103%) and intraday imprecision (<16.0%) across the range of concentrations studied, except for the recoveries at 3.0 μg/L (86.3%) and 5.0 μg/L (80.7%). Comparison of liver (n = 116) and renal (n = 113) patient samples measured by MEIA II against HPLC-tandem mass spectrometry (HPLC-MS/MS) found a mean overestimation of 15.6%. From these comparison data it can be calculated that at values of 5 and 20 μg/L in liver or renal transplant patient samples, measured by HPLC-MS/MS, MEIA II will have the corresponding range estimates of 3.6–7.9 μg/L and 20.9–25.4 μg/L, respectively. No clinically significant difference in results, in terms of overestimation or correlation, was observed between the two transplant groups studied. The MEIA II is an improvement on the previous MEIA I and is suitable for the therapeutic drug monitoring of tacrolimus where HPLC-MS/MS is unavailable.

Tacrolimus (FK506, Prograf®), a potent immunosuppressive agent, was introduced into the clinic in the late 1980s for use as both primary and rescue therapy in patients receiving solid organ transplants (1). The dose-related efficacy and toxicity, narrow therapeutic index, cytochrome P450-mediated drug interactions, and consider-

able interpatient variability in pharmacokinetics of tacrolimus (2) has led to the consensus that therapeutic drug monitoring (TDM)[4] of steady-state trough tacrolimus concentrations is required (3). To date, the most frequently used methodologies for the TDM of tacrolimus have been immunoassay-based, either ELISA or microparticle enzyme immunoassay (MEIA) (4–7). A recent survey reported by Holt and Johnston (8) on the European Tacrolimus Quality Assessment Scheme showed that all but one of the 74 participating members were using immunoassay-based methods. These immunoassays use an anti-tacrolimus monoclonal antibody that recognizes not only the parent drug but also several of its metabolites (9, 10). Several studies have reported that, in different patient groups, these immunoassays show overestimation in tacrolimus concentrations when compared with methods specific for the parent drug (11–15).

Tacrolimus has been shown to exhibit a potency of 100-fold greater than cyclosporin (16), and thus tacrolimus dosage is reduced correspondingly. The resulting blood concentrations (low μg/L) make specific measurement of tacrolimus difficult. However, Gonschior et al. (17) utilized HPLC-mass spectrometry (HPLC-MS) to measure tacrolimus and three of its metabolites simultaneously in blood and urine. Recently, we developed a HPLC-tandem mass spectrometry (HPLC-MS/MS) assay for the analysis of tacrolimus in blood that is specific, sensitive, and rapid (18). Under the guidelines recommended by Büttner (19), both HPLC-MS and HPLC-MS/MS are considered reference procedures for the TDM of tacrolimus. The use of HPLC-MS, however, is limited by its lack of widespread availability and initial capital cost.

[1] The University of Queensland Department of Medicine, Princess Alexandra Hospital, Brisbane, Queensland, Australia 4102.

[2] Department of Clinical Pharmacology, The Queen Elizabeth Hospital, Adelaide, South Australia, Australia 5011.

[3] The University of Queensland Department of Surgery, Princess Alexandra Hospital, Brisbane, Queensland, Australia 4102.

*Address correspondence to this author at: Centre for Clinical and Experimental Therapeutics, First Floor, Lions Clinical Research Building, Princess Alexandra Hospital, Ipswich Road, Brisbane, QLD, Australia 4102. Fax 61 7 3240 5031; e-mail Ptaylor@gpo.pa.uq.edu.au.

[4] Nonstandard abbreviations: TDM, therapeutic drug monitoring; MEIA, microparticle enzyme immunoassay; MS, mass spectrometry; and MS/MS, tandem mass spectrometry.

Recently, a tacrolimus II MEIA (MEIA II) has become available. The lower analytical range of this method, compared with the previous version, MEIA I (7), may provide an alternative to HPLC-MS in the TDM of tacrolimus. In this study, we determined the accuracy and imprecision of the MEIA II, using blood containing weighed-in amounts of tacrolimus. In addition, we evaluated the performance of the MEIA II in the clinical setting against a HPLC-MS/MS assay, using blood samples obtained from two patient groups, liver and renal transplant recipients.

Materials and Methods

MEIA II

The MEIA II was performed on an IMx analyzer® according to the manufacturer's instructions (Abbott Laboratories). Tacrolimus calibrators and controls were used as supplied by Abbott. The IMx analyzer was calibrated (0, 3.0, 6.0, 12.0, 20.0, and 30.0 μg/L) before sample analysis. A series of controls (5.0, 11.0, and 22.0 μg/L) were run with each batch to confirm assay integrity throughout the study. Sample preparation involved blood samples (150 μL) pretreated with a methanol solution containing zinc sulfate and ethylene glycol (150 μL) in 1-mL polypropylene centrifuge tubes. These samples were vortex-mixed and centrifuged for 5 min at 10 600g. The supernatant was dispensed into the respective reaction cells, and the automated analysis was performed.

HPLC-MS/MS PROCEDURE

HPLC-MS/MS analysis was performed according to a modified method of our previously reported assay (20). Briefly, sample preparation consisted of an initial organic solvent precipitation (containing an internal standard: FR900520, a tacrolimus analog, Fujisawa Pharmaceutical Co.) of blood samples (500 μL), followed by a C_{18} solid phase cartridge extraction. The extracted samples were chromatographed using a C_8 column, 2 × 100 mm (Brownlee Laboratories) at ambient temperature. The mobile phase consisted of 200 mL of 40 mmol/L ammonium acetate buffer (pH 5.1) and 800 mL of methanol per liter and was pumped at a flow rate of 400 μL/min. The flow was split postcolumn at a ratio of 1:12 into the mass spectrometer. Mass spectrometric detection was via an electrospray interface using multiple reactant ion monitoring (tacrolimus m/z 821.5→768.4; internal standard m/z 809.5→756.4). For each batch of samples analyzed, a six-point calibration curve (0, 3.0, 6.0, 12.0, 20.0, and 30.0 μg/L) was constructed. A weighted $1/X^2$ regression analysis was used to fit the calibration curves.

ASSESSMENT OF MEIA II AGAINST HPLC-MS/MS

As part of patients' routine clinical care, blood samples were collected by venipuncture 12 h after the last oral dose of tacrolimus, just before the next dose, into tubes containing EDTA. The total number of samples collected from 50 liver and 43 renal transplant patients was 116 and 113, respectively. A maximum of five samples was collected from any one patient. All samples were stored at −18 °C and assayed within 3 months of collection.

STATISTICAL ANALYSIS

To assess the accuracy and imprecision of MEIA II compared with HPLC-MS/MS, three controls (5.0, 11.0, and 22.0 μg/L) were assayed in quadruplicate over 4 days by both methods. Accuracy was calculated as the ratio of the mean concentration (n = 12) over the known "weighed-in" concentration, expressed as a percentage. Imprecision was determined from the analysis of variance, using the methods of Krouwer and Rabinowitz (21). To investigate assay performance at the low end of the therapeutic range, the manufacturer's calibrator B (3.0 μg/L) was analyzed in replicate on 1 day by MEIA II (n = 10) and HPLC-MS/MS (n = 5). The acceptable limit of accuracy was defined as ±15% of the weighed-in concentration and ±20% at the limit of quantitation. Similarly, the acceptable limit of imprecision is defined as CV <15%, except for the limit of quantitation, where it should be <20% (22). Regression analysis and the methods described by Bland and Altman (23) were used to compare the results obtained by the two methods for the liver and renal transplant groups.

Results

A summary of the analytical performances of the MEIA II and HPLC-MS/MS methods at three concentrations (5.0, 11.0, and 22.0 μg/L) is shown in Table 1. The total imprecision of MEIA II was acceptable (<13.0%) across

Table 1. Accuracy and imprecision of MEIA II and HPLC-MS/MS methods for tacrolimus (n = 12).

Method	Tacrolimus concentration, μg/L		Recovery,[a] %	CV,[b] %		
	Weighed-in	Assayed mean		Within-day	Between-day	Total
MEIA II	5.0	4.03	80.7	10.8	5.2	12.0
	11.0	11.3	102.5	7.0	1.0	8.1
	22.0	21.9	99.7	3.4	3.0	4.5
HPLC-MS/MS	5.0	5.03	100.6	3.3	2.0	3.8
	11.0	11.05	100.5	2.5	0.77	2.6
	22.0	21.9	99.5	2.2	0.74	2.3

[a] Recovery is the mean concentration/known concentration × 100%.
[b] Determined by the method of Krouwer and Rabinowitz (21).

the range of controls studied. The recovery of the MEIA II was acceptable at 11.0 μg/L (102.5%) and 22.0 μg/L (99.7%); however, the recovery at 5.0 μg/L (80.7%) was unacceptable. The intraday imprecision and recovery determined at 3.0 μg/L, using the manufacturer's calibrator B, for MEIA II were 15.3% and 86.3%, respectively. These data compare with a total imprecision of <4.0% and a recovery range of 99.5–100.6% for the HPLC-MS/MS method over the range of controls studied. The imprecision and recovery at 3.0 μg/L for the HPLC-MS/MS were 2.5% and 102.2%, respectively.

The tacrolimus concentrations measured by both methods for all the transplant patient samples studied are shown in Fig. 1. The equation for the line of best fit is described by the equation MEIA II = 1.16 ($\pm$ 0.0184) $\times$ HPLC-MS/MS − 0.00561 ($\pm$ 0.163) μg/L ($S_{y|x}$ = 1.12, n = 229). Fig. 2 shows the above data as the difference in tacrolimus concentrations (MEIA II minus HPLC-MS/MS) against the mean tacrolimus concentrations of both methods. The line of best fit is described by the equation MEIA II minus HPLC-MS/MS = 0.179 ($\pm$ 0.0155) $\times$ Mean Concentration − 0.261 ($\pm$ 0.149) μg/L ($S_{y|x}$ = 1.03, mean bias = 1.26 μg/L). From the 95% confidence intervals it can be calculated that concentrations of 5 and 20 μg/L, measured by HPLC-MS/MS, will give corresponding estimates for MEIA II of 3.6–7.9 μg/L and 20.9–25.4 μg/L, respectively. When the data for the two transplant groups were examined independently, little difference, in terms of correlation, was observed. The mean overestima-
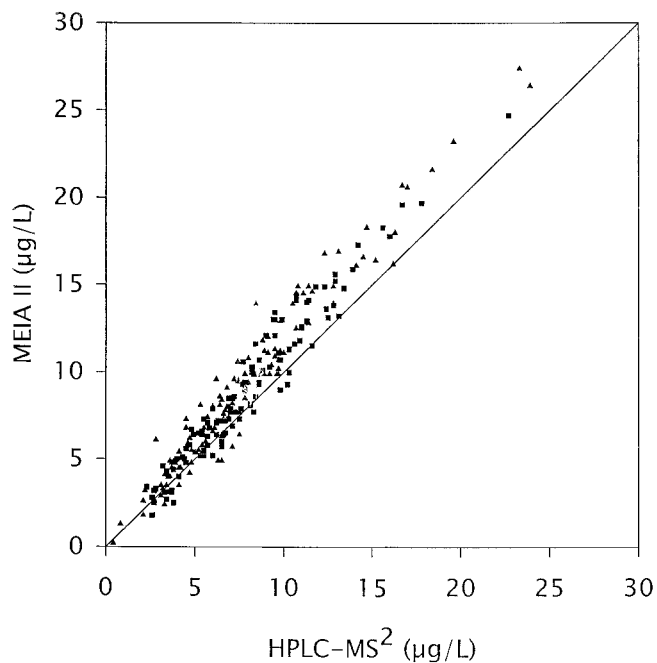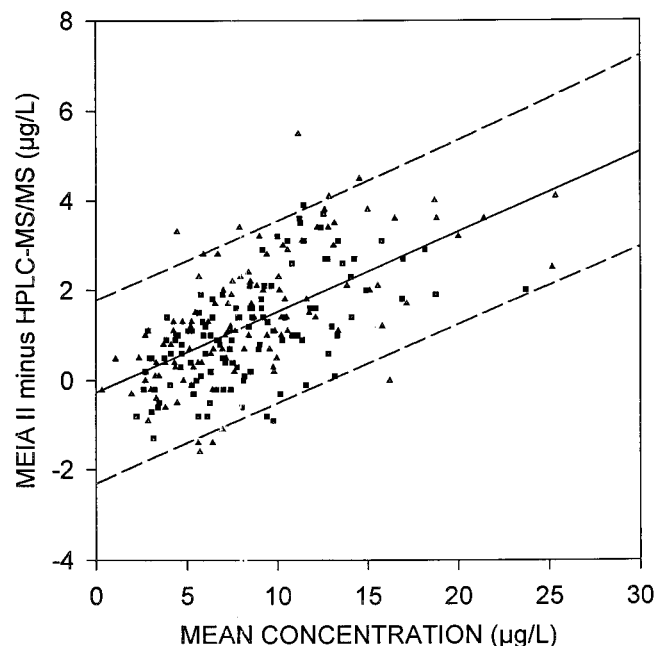

Fig. 2. Difference between the MEIA II and HPLC-MS/MS tacrolimus concentrations compared with the mean tacrolimus concentrations for liver (■, n = 116) and renal (▲, n = 113) transplant patient samples.

The *solid line* represents the line of best fit (MEIA II minus HPLC-MS/MS = 0.179 ($\pm$ 0.0155) $\times$ Mean Concentration − 0.261 ($\pm$ 0.149) μg/L; $S_{y|x}$ = 1.03; mean bias = 1.26 μg/L). The *dashed lines* represent the 95% confidence intervals for the differences.

tions for the liver and renal transplant groups were 13.1% and 18.2%, respectively.

Discussion

Although the recommendations of the Lake Louise International Consensus Conference on Immunosuppressive Drugs called for the use of specific methods in the TDM of tacrolimus *(3)*, the majority of centers use ELISA and MEIA *(8)*. This is partially because accurate measurement of tacrolimus in blood using traditional HPLC detection is not possible due to the drug's lack of intrinsic fluorescence, end absorption in the ultraviolet spectrum *(24)*, and circulating concentrations are low compared with cyclosporin. The development of reliable HPLC-MS methods has provided one means for the specific measurement of tacrolimus *(17, 18)*. The major problem to overcome for the routine use of HPLC-MS is the specialized and expensive instrumentation. The instrumentation required for ELISA and MEIA are less expensive and are found routinely in clinical laboratories. However, the consumable costs of running HPLC-MS analysis is the same or less than traditional HPLC and also less than the cost of the commercial kits for the MEIA II. Finally, the simple sample preparation and rapid turnaround time make the MEIA II very attractive to the clinical laboratory.

The current therapeutic ranges of tacrolimus for liver and renal transplant recipients are in the range of 5–20 μg/L *(25)*. The original MEIA I assay was found to have

Fig. 1. Tacrolimus blood concentrations in liver (■, n = 116) and renal (▲, n = 113) transplant recipients, measured by MEIA II and HPLC-MS/MS).

The *solid line* represents the line of identity (i.e., slope = 1).

insufficient sensitivity to monitor patients with tacrolimus concentrations <5 μg/L *(13)*. Therefore, the development of a MEIA II method with a lower analytical range is of considerable interest to transplantation centers measuring tacrolimus. Our investigation of the analytical performance of the MEIA II, using known weighed-in controls, found the assay to be accurate and precise except for the recovery at 5.0 μg/L (80.7%), although the mean value of 4.0 μg/L obtained for the 5.0 μg/L control was considered acceptable under the manufacturer's guidelines (3.0–7.0 μg/L) *(26)*. Furthermore, the MEIA II assessed using the manufacturer's calibrator B (3.0 μg/L) gave a recovery and imprecision within the guidelines of Shah et al. *(22)* for the limit of quantitation. Overall, the analytical performance of MEIA II, determined using weighed-in controls, was superior to our previously reported evaluation for MEIA I *(15)*.

Several groups have reported increased tacrolimus concentrations in patient samples measured by immunoassays compared with results obtained by specific methods *(11–15)*. In this study, we found that the overestimation was similar for both transplant groups. For the liver and renal transplant groups, the maximum percentages of overestimation (mean) were 47.8% (13.1%) and 117.9% (18.2%), respectively. These data compare with the work of Firdaous et al. *(13)*, in which the reported overestimation for the liver and renal transplant groups ranged from 18% to 48%. The clinical importance of this overestimation in tacrolimus concentrations is yet to be determined, although Braun et al. *(14)* reported a case study of a renal transplant patient with ongoing rejection, in which the difference in concentrations measured by MEIA I (median, 10.5 μg/L), ELISA (median, 7.92 μg/L), and HPLC-MS (median, 2.93 μg/L) could be considered clinically important.

In conclusion, the MEIA II is an improvement on the previously available MEIA I and is suitable for the TDM of tacrolimus where HPLC-MS/MS is not available. The random sample selection for our study was an attempt to obtain a cross-section of our total population; however, determining the clinical importance of the observed overestimation in results will require longitudinal studies with large numbers of patients in all transplant populations.

References

1. Steinmuller DR. FK506 and organ transplantation. Boca Raton, FL: CRC Press, 1994:19–74.
2. Peters DH, Fitton A, Plosker GL, Faulds D. Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drugs 1993;46:746–96.
3. Jusko WJ, Thomson AW, Fung J, McMaster P, Wong SH, Zybler-Katz E, et al. Consensus document: therapeutic monitoring of tacrolimus (FK-506). Ther Drug Monit 1995;17:606–14.
4. Tamura K, Kobayashi M, Hashimoto K, Kojima K, Nagase K, Iwasaki K, et al. A highly sensitive method to assay FK-506 levels in plasma. Transplant Proc 1987;5(Suppl 6):23–9.
5. Wallemacq PE, Firdaous I, Hassoun A. Improvement and assessment of enzyme-linked immunosorbent assay to detect low FK506 concentrations in plasma or whole blood within 6 hours. Clin Chem 1993;39:1045–9.
6. D'ambrosio R, Jusko WJ, Stabler T, Kobayashi M. Improved procedures for enzyme immunoassay for tacrolimus (FK506) in whole blood [Letter]. Clin Chem 1994;40:159–60.
7. Grenier FC, Luczkiw J, Bergmann M, Lunetta S, Morrison M, Blonski D, et al. A whole blood FK 506 assay for the IMx analyzer. Transplant Proc 1991;23:2748–9.
8. Holt DW, Johnston A. Tacrolimus quality assessment [Letter]. Ther Drug Monit 1997;19:243.
9. Iwasaki K, Shiraga T, Nagase Z, Tozuka Z, Noda K, Sakuma S, et al. Isolation, identification, and biological activities of oxidative metabolites of FK506, a potent immunosuppressive macrolide lactone. Drug Metab Dispos 1993;21:971–7.
10. Iwasaki K, Shiraga T, Matsuda H, Nagase K, Tokuma Y, Hata T, et al. Further metabolism of FK506 (tacrolimus). Identification and biological activities of the metabolites oxidised at multiple sites of FK506. Drug Metab Dispos 1995;23:28–34.
11. Gonschior A-K, Christians U, Braun F, Winkler M, Linck A, Baumann J, Sewing K-F. Measurement of blood concentrations of FK506 (tacrolimus) and its metabolites in seven liver graft patients after the first dose by h.p.l.c.-MS and microparticle enzyme immunoassay (MEIA). Br J Clin Pharmacol 1994;38:567–71.
12. Warty V, Zuckerman S, Venkataramanan R, Lever J, Chao J, Mckaveney T, et al. Tacrolimus analysis: a comparison of different methods and matrices. Ther Drug Monit 1995;17:159–67.
13. Firdaous I, Hassoun A, Otte JB, Reding R, Squifflet JP, Besse T, Wallemacq PE. HPLC-microparticle enzyme immunoassay specific for tacrolimus in whole blood of hepatic and renal transplant patients. Clin Chem 1995;41:1292–6.
14. Braun F, Lorf T, Schütz E, Christians U, Grupp C, Sattler B, et al. Clinical relevance of monitoring tacrolimus: comparison of microparticle enzyme immunoassay, enzyme-linked immunosorbent assay, and liquid chromatography mass spectrometry in renal transplant recipients converted from cyclosporine to tacrolimus. Transplant Proc 1996;28:3175–6.
15. Salm P, Taylor PJ, Clark A, Balderson GA, Grygotis A, Norris RLG, et al. High-performance liquid chromatography-tandem mass spectrometry as a reference for analysis of tacrolimus (FK506) to assess two immunoassays in patients with liver and renal transplants. Ther Drug Monit 1997;19:694–700.
16. Kino T, Inamura F, Sakai K, Nakahara K, Goto T, Okuhara M, et al. Effect of FK-506 on human mixed lymphocyte reaction in vitro. Transplant Proc 1987;19(Suppl 6):36–9.
17. Gonschior A-K, Christians U, Winkler M, Schiebel HM, Linck A, Sewing K-F. Simplified high-performance liquid chromatography-mass spectrometry assay for measurement of tacrolimus and its metabolites and cross-validation with microparticle enzyme immunoassay. Ther Drug Monit 1995;17:504–10.
18. Taylor PJ, Jones A, Balderson GA, Lynch SV, Norris RLG, Pond SM. Sensitive, specific quantitative analysis of tacrolimus (FK506) in blood by liquid chromatography-electrospray tandem mass spectrometry. Clin Chem 1996;42:279–85.
19. Büttner J. Reference materials and reference methods in labora-

tory medicine: a challenge to international cooperation. Eur J Clin Chem Clin Biochem 1994;32:571–77.

20. Taylor PJ, Hogan NS, Lynch SV, Johnson AG, Pond SM. An improved method for the therapeutic drug monitoring of tacrolimus (FK506) using tandem mass spectrometry [Technical Brief]. Clin Chem 1997;43:2189–90.

21. Krouwer JS, Rabinowitz R. How to improve estimates of imprecision. Clin Chem 1984;30:290–2.

22. Shah VP, Midha KK, Dighe S, McGilveray IJ, Skelly JP, Yacobi A, et al. Analytical methods validation: bioavailability, bioequivalence, and pharmacokinetic studies. J Pharm Sci 1992;81:309–12.

23. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307–10.

24. Tanaka H, Kuroda A, Marusawa H, Hashimoto M, Hatanaka H, Kino T, et al. Physiochemical properties of FK-506, a novel immunosuppressant isolated from Streptomyces tsukubaensis. Transplant Proc 1987;5(Suppl 6):11–6.

25. McMaster P, Mirza DF, Ismail T, Vennarecci G, Patapis P, Mayer AD. Therapeutic drug monitoring of tacrolimus in clinical transplantation. Ther Drug Monit 1995;17:602–5.

26. Abbott Laboratories. Reagent pack insert for Tacrolimus II. Abbott Park, IL: Abbott Laboratories, 1996:4.