Comparison of Tacrolimus Concentrations Measured by the IMx Tacrolimus II vs the PRO-TRAC II FK506 ELISA Assays, Zhimin (Tim) Cao,1 Mark W. Linder,1* Anthony W. Jeovas,2 Glenda Brown,1 and Roland Valdes, Jr.1
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Contemporary practice in the therapeutic monitoring of the immunosuppressant tacrolimus has required increasingly rapid and more sensitive assays. To this end, the two most commonly used immunoassay formats, IMx Tacrolimus II (Tacrolimus II) and PRO-TRAC II™ FK506 (ProTrac II) have undergone extensive revision, leading to changes in the measured drug concentration relative to the original assay format (1–3). Multiple studies have compared the original microparticle enzyme immunoassay (MEIA) and second-generation Tacrolimus II methods [e.g., Refs. (2, 3)], one report has compared the original MEIA and the second-generation ProTrac II method (1), and two preliminary reports have compared the ProTrac II method with the Tacrolimus II method (4, 5). In each of these reports, the ProTrac II method consistently yielded lower tacrolimus concentrations than the Tacrolimus II assay.

The aim of this study was to further characterize the correlation between the Tacrolimus II method and the ProTrac II ELISA. Tacrolimus II controls were kindly provided by Abbott Laboratories (Abbott Park, IL). Pure tacrolimus powder was a courtesy of Fujisawa USA (Chicago, IL).

Tacrolimus stock solutions in whole blood at concentrations of 5.0, 10.0, and 20.0 μg/L were prepared from tacrolimus powder. Tacrolimus powder was initially dissolved in methanol, followed by sequential dilution with drug-free human whole blood. Tacrolimus assays were performed according to manufacturers instructions. Correlation data were analyzed by Deming regression [Medsnap program (6)]. Differences between the two methods were analyzed by the methods of Bland and Altman (7, 8).

Fifty-five whole blood specimens from 39 transplant patients [heart (n = 9), lung (n = 9), kidney (n = 7), liver (n = 10), and bone-marrow (n = 4); human studies approval UHSC 35-97] were initially assayed for tacrolimus with the ProTrac II method (PRO-TRAC II FK506 Kit). The specimens were then kept at −20°C until...
assayed (<20 days) for tacrolimus concentration with the Tacrolimus II method. Deming regression analysis of patient results yielded the mathematical best-fit of Tacrolimus II = 1.18 (ProTrac II) + 2.19 μg/L (SE); slope = 0.089 [95% confidence interval (CI), 1.0015–1.3615]; y-intercept = 0.833 (95% CI, 0.5192–3.8585); Fig. 1A. The magnitude of the differences between the methods was estimated by the difference against average method of Bland and Altman (7) revealed both a constant and proportional bias for each of these data sets by log of differences vs log of average concentration yielded a mean bias (95% CI) of 13% (−4.5% to −21%) for patient samples and 17% (−9.6% to −23.2%) for drug-free whole blood. The bias between the methods for recovery from drug-free whole blood did not differ from the bias observed for patient specimens (P > 0.74).

When whole blood specimens from tacrolimus-treated patients were used, the correlation of the two methods demonstrated that tacrolimus concentrations measured with the Tacrolimus II method are consistently higher than those measured with the ProTrac II method with a constant bias of 2.19 μg/L and a proportional bias of 18% (P < 0.05). Analysis of differences by the method of Bland and Altman (7) revealed a mean difference of 3.75 ± 1.96 μg/L. Correcting for proportional bias by logarithmic transformation of the data demonstrated that the mean difference in tacrolimus measurements between these methods was 16% (95% CI, 0–29.2%). Murthy and Soldin (5) reported an ordinary least-squares regression of Tacrolimus II = 0.89 (ProTrac II) + 3 μg/L. In agreement with these data, the constant bias is consistently between 2 and 3 μg/L. One reason for the discrepancy in the slope of the regression line may be attributable to differences in the concentration ranges included in each study. Murthy and Soldin included primarily samples with <10 μg/L (S. Soldin, personal communication), whereas for the data presented here, the majority of samples measured >10 μg/L.

Wallenmacq et al. (3) demonstrated that the Tacrolimus II assay yielded lower results than Tacrolimus I with a mean difference of 1.26 ± 2.63 μg/L, and Tredger et al. (2) found similar differences (1.02–2.05 μg/L) between these methods. Salm et al. (9) reported that the Tacrolimus I method yielded a positive bias of 1.8 μg/L vs HPLC-tandem mass spectrometry (MS/MS). Thus, one would
predict that the Tacrolimus II assay would agree well with HPLC-MS/MS. However, Cogill et al. (10) found that the Tacrolimus II method yielded ~16% overestimation of the true tacrolimus concentration in patient samples based on comparison to HPLC-MS/MS. Our data support the literature with respect to overestimation of tacrolimus concentrations in patient samples by the Tacrolimus II assay.

In the first report of the ProTrac II method using proteolytic extraction, MacFarlane et al. (1) reported that the ProTrac II method yielded ~20% lower values than the predecessor ELISA that used methanol extraction. Salm et al. (9) reported that the ELISA method (methanol extraction) yielded results with minimal bias of <0.2 µg/L relative to HPLC-MS/MS. Comparable data for the ProTrac II method currently in clinical use are not available other than one report from MacFarlane et al. (11), who demonstrated comparable results between ProTrac II and HPLC-MS/MS. However, insufficient methodologic detail is given for critique of the data. Taken together, the available data would suggest that the ProTrac II method would yield lower results than HPLC-MS/MS, which is consistent with our recovery data reported here.

In comparison with Tacrolimus I, the ProTrac II method yielded >20% lower values than the Tacrolimus I assay on patient samples (mean difference, 4.2 ± 2.6 µg/L; P <0.05) (1). However, although the Tacrolimus II assay yields lower concentrations than Tacrolimus I (~2 µg/L on average), our data clearly demonstrate that a positive bias persists between the current ELISA and MEIA methods.

The principal source of discrepancy between the MEIA I and ELISA I assays has been attributed to differences in the extraction procedures (I, 11), potentially related to differences in extraction efficiency of tacrolimus and cross-reacting metabolites. To address this possibility, we compared the recovery of pure tacrolimus introduced into samples containing tacrolimus and metabolites and drug-free whole blood. These results yielded recoveries of 112% ± 22% and 108% ± 2% from patient samples and drug-free samples, respectively, for the Tacrolimus II assay and 74% ± 15% and 71% ± 8%, respectively, for the ProTrac II assay. Although the results of this study cannot directly rule out differences in extraction efficiency of tacrolimus and cross-reacting metabolites, the consistent bias we observed in the absence and presence of metabolites [−13% (95% CI, −4.5% to −21%) for patient samples vs −17% (95% CI, −9.6% to −23.2%) for drug-free samples with added tacrolimus] argues against potential differences in metabolite extraction efficiency.

In summary, our data demonstrate differences in measured tacrolimus concentrations as great as 7.6 µg/L between the two most commonly used immunoassays for therapeutic monitoring of tacrolimus. Consistent with the current literature, neither immunoassay method is entirely accurate over the range of concentrations measured. The Tacrolimus II method overestimates true tacrolimus concentrations, and in contrast to current reports, we found that the ProTrac II ELISA underestimates the concentration of tacrolimus by ~25% on average.

Based on the differences in tacrolimus concentrations measured using the two methods, laboratories should clearly communicate with physicians regarding method-based bias and ensure that individual patients are not monitored by the two methods simultaneously. In addition, these data should be carefully evaluated with respect to the assay used in clinical studies that have defined the currently accepted therapeutic range.

References

Detection of Dietary Antioxidant Phenolic Compounds in Human LDL, Rosa M. Lamuela-Raventos,1 María-Isabel Covas,2,3 Montserrat Fitó,1 Jaume Marrugat,1 and M. Carmen de la Torre-Boronat1 (1 Departament de Bromatologia i Nutrició, Facultat de Farmàcia, Universitat de Barcelona, 08028 Barcelona, Spain; 2 Unitat de Lipids i Epidemiologia Cardiovascular, Institut Municipal d’Investigació Mèdica (IMIM), Carrer Doctor Aigüader, 80, 08003 Barcelona, Spain; 3 Laboratori de Referència de Catalunya, 8027 Spain.; 4 Laboratori de Refere`ncia de Catalunya, 8027 Spain.; 5 Laboratori de Refere`ncia de Catalunya, 8027 Spain.; * author for correspondence: fax 34-93-2213237, e-mail mcovas@imim.es)

There is growing interest in the role of phenolic compounds in the diet as antioxidants. Epidemiological studies support a relationship between the consumption of phenolic rich food products (I, 2) and a low incidence of coronary heart disease. Strong evidence exists that oxidation of LDL lipids is a risk factor for atherosclerosis and coronary heart disease (3). Oxidation of LDL appears to occur predominantly in arterial intima in microdomains