Comparison of Blood Tacrolimus Concentrations in Liver and Kidney Transplant Recipients using ACMIA and MEIA Immunoassays

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Abstract

Background: Published data on the performance of the new Dade Behring antibody conjugated magnetic immunoassay (ACMIA) for tacrolimus determination are scarce. The aim of this study was to compare the results obtained using the ACMIA and Abbott microparticle enzyme immunoassay (MEIA), which is the most widely used method for therapeutic tacrolimus monitoring.

Methods: Trough tacrolimus concentrations were determined in 305 blood samples from kidney (n=138) and liver (n=167) transplant recipients using the ACMIA and MEIA immunoassays. The MEIA results were corrected for hematocrit values lesser than 30% and higher than 40% (Hermida et al. Clin Lab 2005; 51: 43–45).

Results: The obtained ACMIA within- and between-run variation coefficients (<10.8%) were acceptable. In the comparison between ACMIA and MEIA results in the blood samples studied, the regression equation was: ACMIA=1.02MEIA+0.29 (r=0.912, p<0.001), with an acceptable difference between the means (8.13±0.53 ng/mL vs. 7.62±0.50 ng/mL). However, in accordance with the well-established interference of the hematocrit on the MEIA results, a highly significant negative correlation between the MEIA/ACMIA ratio and the hematocrit values was obtained (r=-0.585, p<0.001). When the MEIA results were corrected according to the hematocrit (MEIAHtC), the regression with ACMIA levels was: ACMIA=1.08MEIAHtC-0.09 (r=0.926, p<0.001). This equation was analogous to that obtained between ACMIA and MEIA tacrolimus concentrations in the 164 blood samples with hematocrit of 30–40%.

Conclusions: ACMIA is an acceptable option for therapeutic tacrolimus monitoring, with an important decrease in technician time in relation to the widely used MEIA.

Introduction

The immunosuppressant tacrolimus is widely used for the prevention of organ transplant rejection, and due to its narrow therapeutic range and its considerable pharmacokinetic variability, whole blood trough concentrations are routinely monitored. High performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) is a more sensitive and specific procedure than the current commercial immunoassays, for which a variable overestimation of tacrolimus concentrations, attributed to the cross-reactivity of circulating metabolites, has been reported (1–3); however, the Abbott microparticle enzyme immunoassay (MEIA) is still the most widely used method in the clinical practice. Recently, Dade Behring

has developed an antibody conjugated magnetic immunoassay (ACMIA), without the need for the blood sample pre-treatment step, and which is faster than other immunoassays for therapeutic tacrolimus monitoring. In the Tacrolimus International Proficiency Testing Scheme survey, the number of laboratories using ACMIA has grown quickly, currently standing at 13% of the total, compared to 41% who use the MEIA (June, 2007).

The ACMIA method for cyclosporine determination has been evaluated previously (4–8); however, published data on the performance of this new heterogeneous immunoassay for tacrolimus monitoring are scarce (9). In the present study we compared the tacrolimus concentrations obtained using ACMIA and MEIA in blood samples from liver and kidney transplant recipients.

Material and methods

Trough tacrolimus concentrations were determined in 305 blood samples (167 from liver and 138 from kidney transplant recipients) collected in Vacutainer® tubes containing K_3EDTA as anticoagulant. The quantification of tacrolimus was carried out using the ACMIA-Flex® immunoassay (lot BA8012) in a Dimension Xpand Plus analyzer (Dade Behring) and MEIA in an Abbot IMx analyzer (Abbot Laboratories). MEIA results were corrected according to the hematocrit as previously described by Hermida et al. (10), assuming that, in the case of a hematocrit lower than 30% and higher than 40%, a linear error would be produced, with a positive or negative value respectively of 3% per hematocrit unit.

The Statgraphics package (v 5.0) was used for the statistical analysis of the data, and the Kolmogorov-Smirnov test was applied to check for normality. Pearson's correlation coefficient was used when the data had Gaussian distributions; otherwise, Spearman's correlation coefficient was used. The regression study was made using the Passing-Bablock method, using ma68 as a dispersion measure. Tacrolimus concentrations were also compared using the Eksborg's difference plot (11). The results were expressed as mean±SD (median). According to the consensus validation criteria of analytical methods for the quantitative determination of drugs and their metabolites in a biological matrix (12,13), the acceptance criteria are a variation coefficient of no more than 15% for imprecision, and a deviation of no more than 15% from the nominal value for accuracy.

Results

Table 1 shows the results obtained for within-run and between-run imprecision for the determination of tacrolimus by ACMIA, using the Tac/CsA Immunosuppressant Controls (More Diagnostics Inc, Los Osos, CA, USA) provided by Dade Behring. In no case did the variation coefficients exceed the acceptance value of 15% (12,13), and the differences between the assigned values with those obtained were lower than 15%.

Table 1. Imprecision of the tacrolimus determination using ACMIA

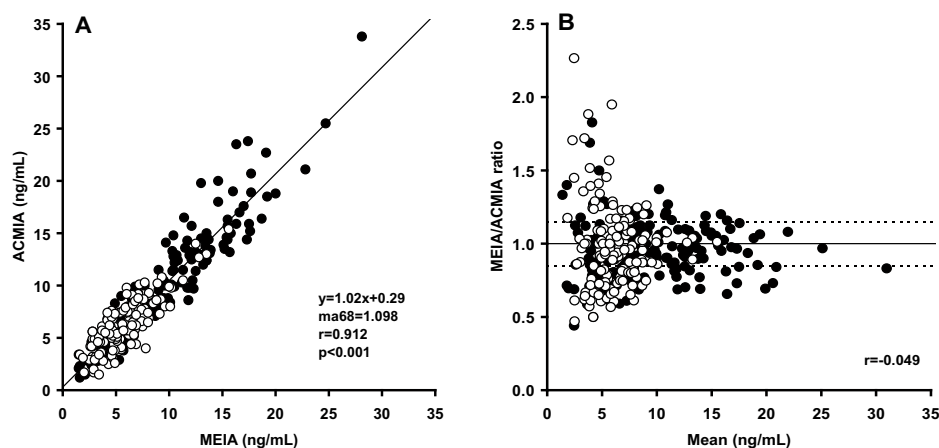|	Mean±SD (ng/mL)	CV (%)
Within-run (n=15)		
Control 1	6.76±0.39	5.8
Control 2	12.80±0.45	3.5
Control 3	16.03±0.97	6.0
Between-run (n=14)		
Control 1	6.28±0.57	9.0
Control 2	12.39±1.34	10.8
Control 3	15.71±1.24	7.9

Figure 1. Passing-Bablock regression plot (A) and Eksborg difference plot (B) for the tacrolimus blood concentrations measured with ACMIA and MEIA in kidney (○) and liver (●) transplant recipient patients. The dotted lines correspond to the limits of the acceptance criterion for deviation.

The correlation and regression between the concentrations of tacrolimus obtained using ACMIA and MEIA in the total number of blood samples studied are shown in Figure 1A. The comparison of the results using Eksborg's difference plot (11) is shown in Figure 1B, with 55% of the cases having a deviation between the levels obtained by both immunoassays of less than 15%. Although no significant correlation was found between the MEIA/ACMIA ratio and the mean concentration of tacrolimus (Figure 1B), in the first-order partial correlation between these variables, keeping the hematocrit constant, statistical significance was achieved ($r=-0.354$, $p<0.001$).

In the samples from liver transplant recipients (n=167), a regression equation was found: ACMIA=1.01MEIA+0.38 (ma68=1.13 ng/mL, $r=0.950$, $p<0.001$) and

Table 2. Correlation and regression between blood tacrolimus concentrations using ACMIA (y) and MEIA (x) for different ranges of hematocrit.

Hematocrit	n	ACMIA (ng/mL)*	MEIA (ng/mL)*	Regression	r	ma68
<30%	71	9.35±1.24 (8.5)	10.03±1.13 (9.0)	y =1.07x-1.68	0.955	0.88
30-40%	164	8.29±0.77 (6.7)	7.68±0.68 (6.6)	y =1.08x-0.15	0.929	0.92
>40%	70	6.52±0.68 (6.5)	5.04±0.59 (4.7)	y =1.18x+0.49	0.936	0.64
Total	305	8.13±0.53 (7.2)	7.62±0.50 (6.6)	y =1.02x+0.29	0.912	1.10

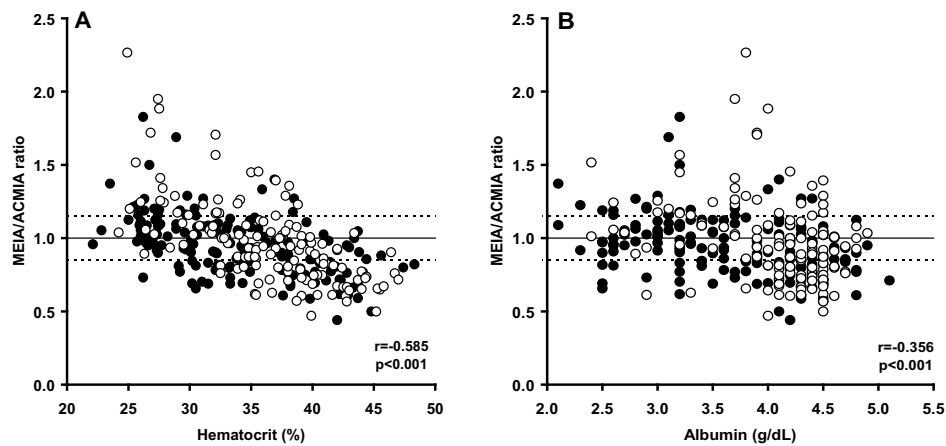*The results are expressed as mean±SD (median).

Figure 2. Correlation of the tacrolimus MEIA/ACMIA ratio with the hematocrit values (A) and serum albumin concentrations (B) in kidney (○) and liver (●) transplant recipient patients. The dotted lines correspond to the limits of the acceptance criterion for deviation.

in those from kidney transplant recipients (n=138): ACMIA=1.04MEIA+0.08 (ma68=0.98 ng/mL, r=0.789, p<0.001).

Figure 2A shows how the MEIA/ACMIA ratio presented a highly significant negative correlation with the hematocrit (p<0.001), with Table 2 showing the correlation and regression between the tacrolimus concentrations obtained using both immunoassays for hematocrit values <30%, 30–40% and >40%. The deviation between the ACMIA and MEIA means (medians) was only higher than 15% in the group of samples with a hematocrit higher than 40%. Correcting the MEIA tacrolimus concentrations according to the hematocrit (MEIAHtc) (10), the regression equation found with ACMIA levels was: ACMIA=1.08MEIAHtc–0.09 (ma68=0.98 ng/mL, r=0.926, p<0.001), with an increase in the number of cases (66%) with de-

viations of less than 15% between the results of both immunoassays. This regression equation is very similar to that found for ACMIA and MEIA concentrations in the group of blood samples with hematocrit of 30–40% (Table 2).

Figure 2B shows the negative significant correlation found between the MEIA/ACMIA ratio and serum albumin (p<0.001); however in the first-order partial correlation between these variables, keeping hematocrit constant, both in the total group (r=0.064), and in the groups of kidney (r= 0.080) and liver (r=-0.027) transplant recipients, the statistical significance was not achieved.

Twenty-eight blood samples, whose tacrolimus concentration had been determined by ACMIA (y), were kept frozen for 5 days and then thawed and centrifuged, once again determining the tacrolimus concentration in the supernatants using this immunoassay (x). The regression equation found was: y=0.95x+0.24 (ma68=0.701 ng/mL, r=0.925, p<0.001), with tacrolimus levels of 7.16±4.20 ng/mL (6.50 ng/mL) and 7.26±4.51 ng/mL (6.20 ng/mL) respectively.

Discussion

The within-and between-run variation coefficients obtained for the determination of tacrolimus in the control material using the ACMIA (Table 1), were acceptable according to the validation criteria used (12,13). Likewise, the correlation coefficient found between the results for ACMIA and MEIA may be considered as satisfactory (Figure 1A). With respect to the lower correlation coefficient obtained in the group of kidney transplant patients (r=0.789, p<0.001), it should be taken into account that they had a lower range of tacrolimus concentrations than the liver transplant patients group, in most cases were <10 ng/mL, and with a higher relative dispersion between the ACMIA and MEIA values (Figure 1B). As regards to the differences between the means (medians) obtained using ACMIA and MEIA, these were acceptable according to the criteria used (12,13) except in the group of samples with a hematocrit higher than 40%, in which the difference was higher than 15% (Table 2).

The effect of the hematocrit on the relationship between tacrolimus concentrations using MEIA and ACMIA methods, was not previously considered by Griffey et al (9). The highly significant negative correlation between the MEIA/ACMIA ratio and the hematocrit (r=-0.585, p<0.001) is analogous to that found previously on comparing the tacrolimus concentrations obtained by MEIA and the enzyme multiplied immunoassay technique (EMIT) (15). As a low hematocrit interference on the ACMIA has been described (14), this fact may be due to the widely documented interfering effect of hematocrit values outside of the range between 30% and 40% on the MEIA results (10,14–17), although the squared r (determination coefficient) is only 0.342. For the total number of samples studied (n=305), when the MEIA levels were corrected according to the hematocrit (10), the regression equation of MEIAHtC with ACMIA concentrations was highly similar to that obtained between MEIA and ACMIA results in the 164 samples with hematocrit of 30–40%.

In 55% of the cases the deviation between the ACMIA and MEIA results was lower than 15%, which increased to 66% after correcting the MEIA results according to the hematocrit. As the blood samples were taken from liver and kidney transplant recipient patients during initial and maintenance therapy, a tacrolimus therapeutic range of 5.0–15.0 ng/mL was considered (18), and in 83% of the cases, ACMIA and MEIA results were concordantly classified in the subtherapeutic, therapeutic and supratherapeutic ranges. When the MEIA concentrations were corrected according to the hematocrit (10), this concordance between ACMIA and MEIAHtC results was in 91% of the cases. Consequently the diagnostic efficiency of ACMIA and MEIA (preferably MEIAHtC) appears similar.

Many transplant centers are now administering low-dose regimens that lead to blood tacrolimus levels lower than 5.0 ng/mL, and from Figure 1B is clear that the variation in the MEIA/ACMIA ratio increase dramatically bellow this tacrolimus concentration. Although at low concentrations a greater analytical imprecision for both immunoassays should be considered, is interesting to emphasize that the larger part of cases with MEIA/ACMIA ratio greater than 1.5 correspond to samples with hematocrit lower than 30%, and with MEIA/ACMIA ratio lower than 0.5 correspond to samples with hematocrit greater than 40%, as may be see in Figure 2A.

A significant negative correlation between the MEIA/ACMIA ratio and serum albumin concentration was found (p<0.001), however, in the first-order partial correlation between these variables, keeping the hematocrit constant, statistical significance was not achieved, either in the total group or in the groups of kidney or liver transplant recipients. We consider this data to be of interest, as it has been recently described that the metabolic enzyme activity of the cytochrome P450 system and the total clearance of hepatically metabolized drugs show a significant correlation with the serum albumin concentration (19). Particularly in the case of the liver transplant recipients, the serum levels of albumin are modulated preferably by their hepatic function, and consequently the tacrolimus metabolization rate does not appear to be capable of introducing an additional source of variation in the relationship between the ACMIA and MEIA results. In fact, the positive bias (slope=1.16) reported for ACMIA and MEIA with respect to HPLC/MS/MS were similar (9), suggesting that both immunoassays present analogous cross-reactivity to the various tacrolimus metabolites. However, in accordance with a recent analyse data from the International Tacrolimus Proficiency Testing Scheme, the differences between the results provided by some immunoassays and HPLC for patient samples compared with spiked samples, would be also influenced by immunoassay calibration inaccuracies (20).

The mean tacrolimus concentration determined by ACMIA in the supernatants of some blood samples that were frozen, and then thawed and centrifuged, was analogous to that found prior to freezing the samples (7.26±4.51 ng/mL vs. 7.16±4.20 ng/mL). This suggest that in the ACMIA methodology, which does not require the previous extraction, precipitation and centrifuging of the samples, the remnants of erythrocyte and leukocyte membranes do not affect the quality of the results.

In conclusion, the ACMIA, a newly-developed immunoassay for tacrolimus de-

termination without the manual pre-treatment of blood samples, may offer a valid alternative to the most widely used MEIA for therapeutic monitoring of this immunosuppressant agent with a significant decrease in technician time.

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