Measurement of Tacrolimus- Does The Choice of Anticoagulant Matter?

Carla R Michalska, Elizabeth W Shiferaw, Atholl Johnston and David W Holt

Analytical Unit, St George's Hospital Medical School, University of London, Cranmer Terrace, London SW17 0RE, England, UK

Background:

Therapeutic drug monitoring (TDM) of the immunosuppressive drug tacrolimus as a guide to administration has become widely accepted practice as the administration must be individualised for each patient[1]. Immunosuppressive drugs are regarded as critical dose drugs due to the narrow therapeutic range between desirable and undesirable effects. The measurement of tacrolimus concentration has been associated with methodological problems related to the choice of sample matrix (blood, plasma and anticoagulant), specificity of immunoassays for the measurement of the parent drug and calibration inaccuracies. Comparative studies such as the International Tacrolimus Proficiency Testing Scheme[2] aim to document the performance of the different assays available when used to measure this drug in samples containing known amounts of the drug and in samples collected from the patients prescribed the drug.

Matrix effects have previously been seen between plasma and blood samples for the immunosuppressive drug ciclosporin. There has also been interest in the interference of anticoagulants and other preservatives causing matrix effects[3]. EDTA-blood is the preferred choice of sample matrix used to measure tacrolimus in whole blood, but citrate blood is often the anticoagulant used by blood banks.

Aim:

To investigate if there is a matrix effect between the two commonly used anticoagulants, EDTA and citrate in whole blood samples when measuring tacrolimus. Heparin has previously been found to form clots during the sample pre-treatment stage of immunoassays[4] and, therefore, has been excluded from the study as it is already known that it interferes with the tacrolimus measurement.

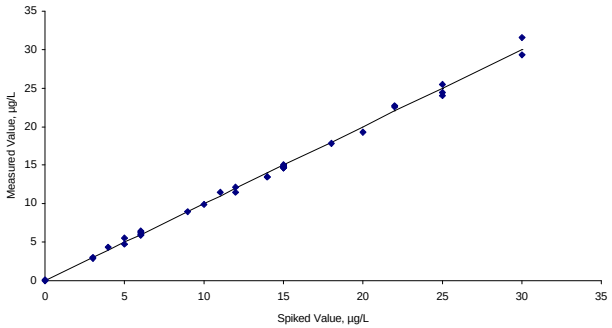

Figure 1. Mean HPLC tacrolimus measurement versus nominal concentration added to 34 blank blood samples.

Methods:

Data from the International Tacrolimus Proficiency Testing Scheme[2] for the period February 2003 to September 2004 were examined. All the data from samples spiked with the drug within this period were plotted to analyse spiking accuracy. In the absence of a reference method, spiking accuracy was assessed by comparison with the HPLC method mean. The nominal tacrolimus concentrations of the samples were (number of aliquots if >1) 0 (2), 3 (2), 4, 5 (2), 6 (5), 9, 10, 11, 12 (2), 14 (2), 15 (6), 18, 20, 22 (2), 25 (3), 30 (2)µg/L. The scheme supplies three blinded samples of blood each month. Typically, two are drug free human whole blood samples with either citrate or EDTA-anticoagulant spiked with tacrolimus to a nominal concentration and the third is a pooled sample of whole blood from patients receiving the drug following organ transplantation. Five spikes were selected from the period February 2003 to September 2004 to provide data for the comparison of citrate and EDTA treated blood. Data were analysed with respect to the analytical technique used.

Data from this study were based on the following samples:
Ten aliquots from tacrolimus-free blood to which known amounts of the drug had been added to five EDTA-anticoagulated and five citrate-anticoagulated blood samples. The nominal tacrolimus concentrations of the samples were 30, 25, 12, 6 (3 x within-assay precision) and 5µg/L.

Data from three methods were suitable for evaluation during the study period. These were HPLC with mass-spectrometric detection (high specificity), IMx immunoassay from Abbott and EMIT immunoassay from Dade Behring. There were too few data for meaningful statistics for an ELISA assay from DiaSorin. Statistical analysis of these spiked samples included the measured value versus measured citrate-HPLC value and % difference from the measured citrate-HPLC value were calculated for each method. Data are displayed as Box and Whisker plots. The measured mean concentration for both EDTA and citrate samples for the five nominal spike concentrations vs nominal concentration were calculated. Data was displayed as a bar chart. An analysis of variance was also performed on the data.

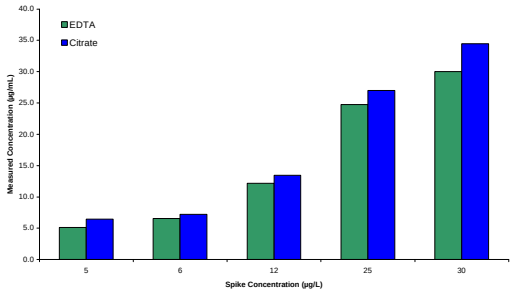

Figure 2. Calculated mean values for five nominal spike concentrations (5, 6(x3), 12, 25, 30µg/L) plotted for both citrate and EDTA-treated blood samples.

Results:

Spiking accuracy during this 20 month period is illustrated by a graph of the results for the weighed in value, against the measured value obtained by HPLC, Figure 1. There was very good agreement between the two variables. Analysis of variance for all the assay methods showed a positive bias when citrate-treated samples were analysed, the mean values calculated for each spike were plotted for both citrate and EDTA, Figure 2. The percentage increase for tacrolimus in citrated samples compared to EDTA were calculated; the bias was 4.6%, HPLC (n=23) 24.4%, IMx (n=190) and 9.5%, EMIT (n=57).

The analysis of variance for the within-assay 6µg/L citrated and EDTA anticoagulated samples showed citrate-treated samples gave higher results than those treated with EDTA. The bias was larger using the immunoassays. Expressed as a percentage ratio (95% confidence interval) to the citrate-HPLC values, IMx with citrate was 126% (118-133%), EMIT with citrate was 122% (114-131%) and HPLC with EDTA was 95% (88-103%). The results are displayed as a box and whisker plot in Figure 3.

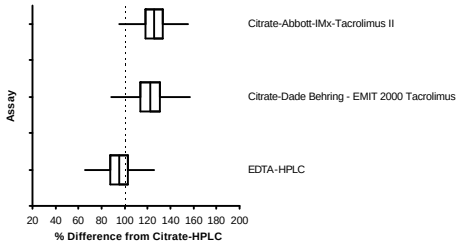

Figure 3. Tacrolimus accuracy by assay method. The box plot shows the percentage inaccuracy for the measurement of tacrolimus in five blank blood samples to which known amounts of tacrolimus had been added.

Conclusions:

The data suggest that the choice of anticoagulant is associated with a sample matrix effect. There was an overestimation of tacrolimus in the samples spiked with the drug using immunoassay and to a much lesser extent using HPLC. The IMx assay was the most affected by the use of citrate in the samples.

References:

1. Holt DW et al. Br J Clin Pharmacol, 52, 61S-73S (2001)
2. International Tacrolimus Proficiency Testing Scheme: www.bioanalytics.co.uk
3. Shaw LM et al. Clin. Chem, 36, 1841-1846 (1990)
4. Beresini MH et al. Clin. Chem. 2235-2241(1993)