CYP3A5 Genotype does not Influence the Measured Blood Concentration of Tacrolimus with the Abbott Immunoassay

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Background:
Previously, we demonstrated that the dose-normalised tacrolimus concentration at 3 months post renal transplantation was associated with the genotype at a single nucleotide polymorphism (SNP) in the CYP3A5 pseudogene. Possession of at least one CYP3A5*1 allele predicts expression of the CYP3A5 gene. This is through linkage disequilibrium with a SNP (at the 6986 position) in the CYP3A5 gene in which individuals with at least CYP3A5*1 (G) allele (wild-type) synthesise the CYP3A5 enzyme and CYP3A5*3 (G) homozygotes do not (genotype AA).

Aim:
To investigate the possibility that CYP3A4 and CYP3A5 produce different metabolite profiles from tacrolimus. If this were the case, the degree of interference in the immunoassay would differ for expressors and non-expressors of CYP3A5. In order to investigate this possibility we compared tacrolimus results obtained using the Abbott IMx immunoassay with those of a high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) assay using whole blood samples taken from expressors (homozygous CYP3A5*1*1 and CYP3A5*1*3 heterozygotes) and non-expressors (homozygous CYP3A5*3*3) of the CYP3A5 gene.

Patient group:
Pre-dose blood samples collected from kidney transplant patients receiving tacrolimus three-months post transplant were analysed. A cohort of 65 patients was grouped into expressors (n=23) and non-expressors (n=42) of CYP3A5.

Method:
Tacrolimus analysis was performed using the Abbott Tacrolimus II MEIA (Microparticle Enzyme Immunoassay) kit and by an LC-MS/MS method. The LC-MS/MS assay was used as the reference method. For direct comparison the same Abbott calibrators and controls were used for both methods to minimise any calibration bias.

The DNA was extracted from whole blood using Qiagen Mini Prep kits. Genotyping was performed using a LightCycler® (Roche) with the FastStart DNA Master Hybridisation Probe PCR method.

Results:
The tacrolimus concentrations, as measured by both methods in CYP3A5 non-expressors and expressors, are shown in Figure 1a and 1b, respectively. A comparison was made of the median ratios of the tacrolimus concentrations obtained using LC-MS/MS and the immunoassay for the expressors and non-expressors. The individual ratios were obtained by dividing the immunoassay result by the LC-MS/MS result.

Using the Mann-Whitney U test we found that there was no significant difference between the median ratios of the two groups (p=0.09) at 95% confidence intervals. The median ratios and interquartile ranges were 1.06, 0.92-1.24 and 0.97, 0.88-1.10 for the expressor and non-expressor groups, respectively.

Conclusion:
Our results suggest that CYP3A5 genotype does not affect tacrolimus measurement using the Abbott immunoassay. Thus, the genetic influence on the pharmacokinetics of tacrolimus is most likely to be related to a genotype-phenotype association rather than an artefact related to the specificity of the assay. Although the presence of metabolites has the potential to interfere with the Abbott immunoassay, we found there was a high degree of concordance between the two assays.

References: