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

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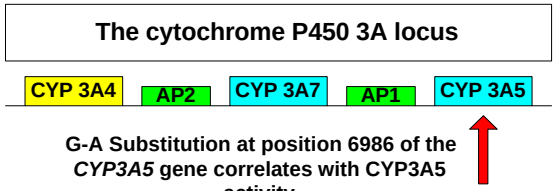
Background:

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 *CYP3AP1* pseudogene. Possession of at least one *CYP3AP1*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*3* homozygotes do not (genotype AA).



Therapeutic drug monitoring of immunosuppressants is well established as an aid to optimising patient management following allograft transplantation. Most laboratories measure ciclosporin and tacrolimus using immunoassays. However, measured values are potentially affected by the presence of circulating metabolites due to the limited specificity of the antibodies employed in these assays.

Ciclosporin is metabolised by the cytochrome P450 3A4 (CYP3A4) and cytochrome P450 3A5 (CYP3A5) enzymes. Studies on the biotransformation of ciclosporin by CYP3A4 and CYP3A5 have demonstrated disperate patterns of metabolite profiles between the two enzymes for this substrate. CYP3A4 catalyses the formation of three primary ciclosporin metabolites, two of which are monohydroxylated (AM1 and AM9) and the third is demethylated (AM4N). CYP3A5 only produces the AM9 metabolite (1). This may have important implications for immunoassay measurements of ciclosporin since the polymorphic expression of the *CYP3A5* gene results in certain individuals not producing the CYP3A5 enzyme (2). In addition, the CYP3A5 enzyme has been shown to have a lower catalytic activity with ciclosporin as compared to CYP3A4. However, the preferential catalysis exhibited by these enzymes is not the same for all substrates.

The preferential production of particular metabolites could occur with tacrolimus. Genetic studies have shown that expressors of *CYP3A5* require significantly larger doses of tacrolimus to attain therapeutic blood concentrations of this drug (3). These studies have been based on blood concentrations determined using the Abbott IMx immunoassay.

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.

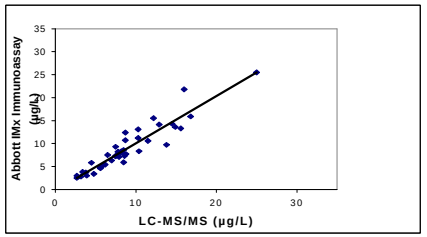
Results:

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.

A



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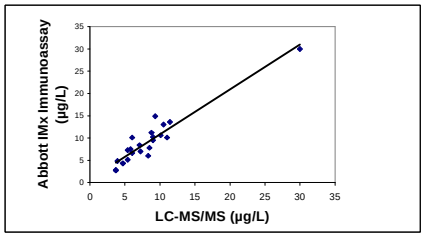

Figure 1 .

Tacrolimus blood concentrations in (a) *CYP3A5* non-expressors (n=42), r^2=0.88 and (b) *CYP3A5* expressors (n=23), r^2=0.90, as measured by Abbott IMx immunoassay and LC-MS/MS. The solid line represents the line of best fit.

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:

1. Aoyama T, et al. J Bio Chem. 1989;18:10388-95.
2. Kuehl P, et al. Nat Genet. 2001 Apr;27(4):383-91.
3.Macphee IA, et al. Transplantation. 2005 Feb 27;79(4):499-502.