

Pharmacogenetics of immunosuppression in organ transplantation

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Dr MacPhee began by outlining the principles of immunosuppression and current immunosuppressive drugs in use. He then presented results about the pharmacogenetic effect of CYP3A5 genotype in predicting blood concentration of the calcineurin inhibitor tacrolimus. This observed effect is the basis of a study currently taking place at St. George's into the use of an individual's CYP3A5 expressor status to plan tacrolimus dosage. Use of CYP3A5 as a predictive tool was compared to results using MDR-1 genotypes, and its effectiveness in predicting drug dosage in patients treated with ciclosporin was discussed. Finally Dr MacPhee described the potential of using pharmacodynamics to predict patient drug response.

The target of immunosuppressive drugs is the lymphocyte. Lymphocytes are unable to distinguish between foreign transplanted tissue and infected self tissue and will therefore raise an immune reaction against solid organ transplants. This can lead to organ failure and in some cases death. Immunosuppression is extremely complicated, as it involves suppressing a critical homeostatic response that can cause patients to become susceptible to infections with pathogens (often those latent in the body) such as viruses and fungi. In addition, each drug has its own potential specific side effects. Therefore, there are many significant complications with both over and under-treatment with immunosuppressive drugs.

Transplant patients receive a range of drugs including a) calcineurin inhibitors (inhibit the T-lymphocyte response) - e.g. ciclosporin and tacrolimus, b) mTOR inhibitors (inhibit the T-lymphocyte response to cytokines) - e.g. sirolimus and c) corticosteroids. All of these drugs are metabolised by the cytochrome p450 system and are substrates for P-glycoprotein.

A significant problem with use of these drugs is the fact they have a narrow therapeutic index (difference between minimum effective dose and maximum non-toxic dose) and wide individual variation between the blood concentration achieved for a given dose. To overcome this therapeutic drug monitoring is currently used for drugs of types a) and b).

The pharmacogenetic effect in patients treated with tacrolimus was originally discovered following the observation that there are major ethnic differences in the dose requirement for the drug. There was found to be an approximately 50% decrease in the dose normalised blood concentration of tacrolimus (blood concentration achieved per 0.1mg/kg dose) with black

patients when compared to other ethnic groups (Asian, Middle-Eastern, Caucasian). It is critical for patients to achieve a blood concentration within the target range very rapidly. Even on day 2 after transplantation a low concentration is associated with a higher rate of rejection.

A SNP was identified within the CYP3A5 gene at the cytochrome P450 3A locus that showed a pharmacogenetic effect within patients treated with tacrolimus. Individuals are defined as CYP3A5 expressors (CYP3A5*1 genotype, either homozygotes or heterozygotes, express functional protein) or non-expressors (CYP3A5*3 homozygotes).

Genetic analysis of a cohort of 220 transplant patients at St. George's identified that 89% of black patients were expressors compared to only 14% of Caucasians. The data was split by ethnic group to identify whether the effect on blood tacrolimus concentration was due to the CYP3A5 genotype or purely to ethnic differences. In Caucasian and south Asian patients a 2-fold reduction in blood concentration was witnessed in expressors, which is consistent with the observation that CYP3A5 genotype influences blood tacrolimus concentration. Unfortunately there were insufficient numbers of patients in the black population to conclude that the initial ethnic difference observed was due to CYP3A5 genetic variation.

The current protocol for tacrolimus dosing involves measuring the patient's blood concentration three times a week and changing the dose by 20% if the achieved concentration falls outside the target range. Approximately 40% of CYP3A5 expressors fail to make target during the first week after a transplant (compared to less than 10% of non-expressors). This is increased to 75% of expressors if data are solely taken from the Caucasian population to confirm that the difference is due to CYP3A5 and not ethnicity. Episodes of acute rejection appear to occur earlier in the CYP3A5 expressor group that may be an effect of insufficient tacrolimus although a larger study is required to confirm this. In addition the reciprocal effect is also witnessed with over 70% of non-expressors having toxic concentrations of tacrolimus present in their blood at some point during their first week of treatment compared to 40% of expressors.

A randomised controlled trial using a pharmacogenetic approach to planning tacrolimus dosing has begun at St. George's. This is based upon the hypothesis that increasing the starting dose of tacrolimus in CYP3A5 expressors will increase the number of patients achieving target blood concentrations early after transplantation. Patients on the transplant waiting list are to be genotyped for CYP3A5, allowing expressors to be identified and then these individuals will be randomised to either the standard or double the standard starting dose of tacrolimus.

Additional genes have been identified that have an effect on tacrolimus blood concentrations. For example, a number of SNPs have been located within the P-glycoprotein (MDR-1) gene. The best studied is C3435T in exon 26. The wild type CC genotype is associated with high levels of P-glycoprotein expression and lower tacrolimus plasma concentrations. Analysing the data by haplotype of 3 linked SNPs in C3435T (including that in exon 26) and also CYP3A5 expressor status indicates that while there may be a small difference between P-glycoprotein genotype and tacrolimus blood concentration it is not of the same magnitude as that for CYP3A5 and is unlikely to be of practical use. Therefore, the study at St. George's is focussing upon the CYP3A5 genotype only.

Research has also been carried out into patients treated with ciclosporin. This drug is metabolised in the same way as tacrolimus but similar blood concentration variations between different CYP3A5 genotypes and the 3 MDR-1 SNPs are not witnessed. Therefore it cannot be assumed that drugs metabolised in the same way are influenced by the same genetic factors.

Finally Dr MacPhee discussed the impact of genotype on pharmacodynamics that is a more speculative area of research. With transplantation there is the unusual situation where genotypes from 2 different individuals are present. For example, in liver transplantation, the enzymes in the donor liver are different to those in the gut. In the very early period post transplantation (1st week) there is a significant effect of P-glycoprotein genotype on drug concentrations. However later, the CYP3A5 genotype of the donor liver (not the recipient tissue) has the dominant effect on efficacy of drug absorption.

There are data to suggest that P-glycoprotein genotype also affects the efficiency of uptake into the lymphocytes (target organ of immunosuppressive drugs) and drug toxicity. One practical example of P-glycoprotein genetics predicting a pharmacodynamic effect comes from paediatric heart transplant patients. These patients were treated with tacrolimus and then steroid treatment was attempted to be withdrawn. 67% of individuals with the high expressor P glycoprotein genotype needed to return to steroid treatment compared to only 38% of low expressors.

Dr MacPhee concluded that from their research two principal points of importance that must be considered when carrying out pharmacogenetic research are: a) in the identification of a gene with potential use as clinical test all confounding factors need to be taken into account so only the effects of the gene of interest are studied and b) not to make the assumption that drugs metabolised in the same way are prone to the same genetic factors influencing their pharmacology.