ELSEVIER

Influence of Pharmacogenetic Polymorphisms in Routine Immunosuppression Therapy After Renal Transplantation

M.J. Herrero, J. Sánchez-Plumed, M. Galiana, S. Bea, M.R. Marqués, and S.F. Aliño

ABSTRACT

Pharmacogenetics is the study of the cause of various individual responses to the same pharmacologic therapy. Genetic alterations in a single nucleotide in the genes responsible for transport and metabolism of an immunosuppression drug may modify patient response. Although pharmacogenetics is of interest, its clinical relevance remains to be demonstrated. The objective of the present study was to evaluate the effect of single-nucleotide polymorphisms (SNPs) in renal transplant recipients and their donors relative to blood concentrations of tacrolimus in the first 2 weeks posttransplantation. Seventy-one blood samples each from renal transplant recipients and their donors were analyzed using a genetic analysis system (MassARRAY; Sequenom, Inc, San Diego, California) in an attempt to characterize the more relevant SNPs of the *ABCB1* and *CYP3A5* genes for correlation with recipient trough concentrations of drug. Two-way analysis of variance and Bonferroni post hoc tests were used. In agreement with theoretical predictions, the wild-type genotype in *ABCB1* SNPs (*CC*) tended to stabilize drug concentrations within the therapeutic range, whereas the *T* variant induced a mean increase in blood concentrations of more than 60%. These findings are in agreement with statistical tests that compared mean concentrations in various recipient-donor populations and found significant differences between them ($P < .001$) in *CC* vs *TT,* and $P < .01$ in *CT* vs *TT*). Donor genotype did not seem to be relevant. However, further studies are required to achieve more robust conclusions.

PHARMACOGENETICS has been used as a powerful tool for understanding variable patient responses to treatment with the same drug. Pharmacokinetics and pharmacodynamics account for some of the variable responses to drugs relative to other parameters including diet, duration of exposure, and interaction with other agents. However, genetic characteristics of each individual, in particular as related to transport, metabolism, and therapeutic targets, are important to the success of therapy.[1] In organ transplantation, the early success rate has greatly improved due to new immunosuppression agents that have enabled a significant reduction in acute rejection episodes. However, after the first year posttransplantation, the success rate is not substantially improved.[2,3] There are solid reasons to believe that some failures are due to genetic variability derived from single nucleotide polymorphisms (SNPs).

A number of genes are believed to be involved in different effects of immunosuppression therapy (www.fda.gov; www.pharmgkb.org). Two genes in particular have demonstrated clear correlations in several studies: *ABCB1* (or *MDR1*), which codes for the transporter P-glycoprotein,[4] and *CYP3A5,* which codes for an extensive drug-metabolizing enzyme of the cytochrome p450 family.[5] Most published studies included patients with particular clinical conditions and not in true clinical settings. The objective of the present study was to seek correlations between blood concentrations of drug and recipient and donor genotypes, to determine whether pharmacogenetics could be a practical tool in the daily management of these patients.

MATERIALS AND METHODS

One blood sample, collected in anticoagulation tubes at routine extraction, was obtained in each of 71 renal transplant recipients and their donors. The DNA was extracted from 200 μL of blood using a commercially available kit based on centrifugation in microcolumns (UltraClean BloodSpin DNA Isolation Kit; MoBio

From Fundación para la Investigación Hospital La Fe, Unidad Farmacogenética, Servicio Farmacia, Valencia, Spain.

Work partially supported by AP-149/8 and GE-007/10 from Conselleria de Sanitat, Generalitat Valenciana.

Address reprint requests to Maria José Herrero Cervera, Fundación para la Investigación Hospital La Fe, Unidad Farmacogenética, Servicio Farmacia, Avda Campanar 21, 46009 Valencia, Spain. E-mail: maria.jose.herrero@uv.es

0041-1345/10/$–see front matter
doi:10.1016/j.transproceed.2010.05.122

Laboratories, Inc, Carlsbad, California). After quantification using a spectrophotometer (NanoDrop Technologies Inc, Wilmington, Delaware) to determine the concentration and purity, DNA was stored at −20°C until use. A genetic analysis system (MassARRAY; Sequenom, Inc, San Diego, California) was used to obtain the genotypes of each sample in the SNPs rs1045642 (3435C>T), rs2032582 (2677G>T/A), and rs1128503 (1236C>T) of the *ABCB1* gene, and in the SNPs rs776746 (6986A>G, *CYP3A5*3*), rs10264272 (267871G>A, *CYP3A5*6*), and rs41303343 (*CYP3A5*7*) of the *CYP3A5* gene. The 71 recipients were all given tacrolimus as the primary immunosuppression drug, at an initial dose of 0.2 to 0.3 mg/kg/24 h. Blood concentration of tacrolimus was measured routinely using a clinical chemistry system (Dimension; Siemens Healthcare, Deerfield, Illinois) to determine the trough level (in nanograms per milliliter). Concentrations from the first and second weeks were determined, and the mean value for each patient, if there was more than 1 value each week, was calculated. The resulting values, 1 for each week, in each recipient were plotted, highlighting the median value in each group. Differences between groups were evaluated using 2-way analysis of variance followed by the Bonferroni correction.

RESULTS

The SNP genotyping of the 142 samples demonstrated frequencies close to those expected from public databases (SNP Database in NCBI site). For the *ABCB1* gene, in rs1045642 (3435C>T), 33% were *CC,* 50% were *CT,* and 17% were *TT;* in rs1128503 (1236C>T), 40% were *CC,* 46% were *CT,* and 14% were *TT;* and in rs2032582 (2677G>T/A), 39% were *GG,* 52% were *GT,* 1% were *AA,* and 8% were *TT.* For the *CYP3A5* gene, in rs776746 (6986A>G, **3*), 82% were *GG,* 17% were *GA,* and 0.5% were *AA;* in rs10264272 (267871G>A, **6*), 98% were *GG,* and 2% were *GA,* and in rs41303343 (**7*), all samples exhibited the wild-type genotype (no duplication).

The correlation of these genotype data with concentration of tacrolimus in the recipient's blood revealed that the wild-type genotype in the 3 *ABCB1* SNPs tended to stabilize drug concentrations in the therapeutic range (10–15 ng/mL) during the first 3 months posttransplantation. The variant genotype increased blood concentrations between weeks 1 and 2 posttransplantation. Fig 1 shows the data for rs1045642, the most relevant SNP in *ABCB1,* according to the literature.[2,3,5] These results were representative of those in 2 other cases. Whereas the *CC* genotype tended to normalize tacrolimus blood concentrations, both the heterozygous (*CT*) and homozygous forms were associated with an increase of more than 60% between the median values of the 2 weeks. These results were in agreement with statistical tests that compared mean values in the various recipient-donor populations, which demonstrated significant differences between the groups ($P < .001$) in *CC* vs *TT,* and $P < .01$ in *CT* vs *TT.* The corresponding data for the SNPs rs776746 and rs10264272 of *CYP3A5* also were consistent with these findings (data not shown), demonstrating a significant increase in drug concentrations in recipients with the variant compared with those with the wild type. The results also seemed to demonstrate that the important genotype in renal transplantation is that of the recipient because the donor genotype had no effect on drug concentrations.

DISCUSSION

To evaluate the importance of pharmacogenetics in transplantation, more accurate knowledge is needed about the significance of the vast number of SNPs in individual patient response to immunosuppression drugs, not only by studying them individually but also by performing more complex studies of SNP groups and haplotypes. Achieving optimal drug concentration is difficult because of large individual differences in drug-metabolizing enzymes and drug transporters. This is particularly difficult to understand in daily clinical practice, in which there is overlap of effects of many other factors and concomitant treatments. Results of the present study confirmed the predicted effect of 5 important SNPs: those in *ABCB1* and *CYP3A5* caused increased tacrolimus blood concentration in the first days posttransplantation, with significant differences between populations. Thus, in theory, recipients carrying these vari-

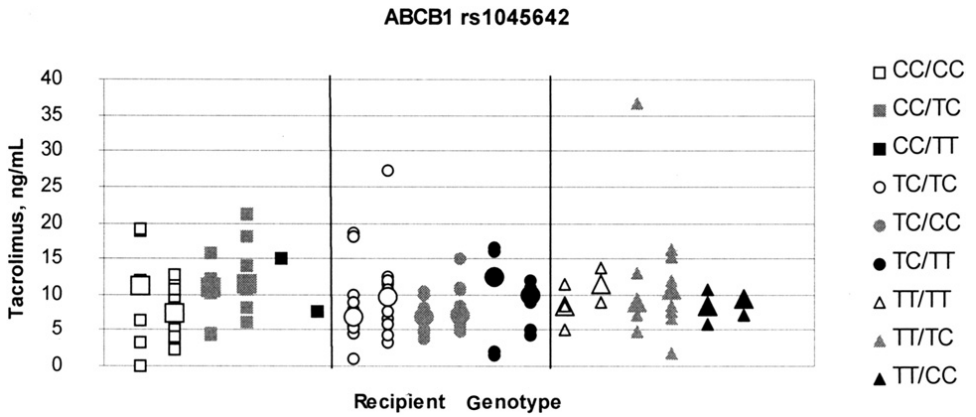

Fig 1. Tacrolimus concentrations in renal transplant recipients. Recipients are shown according to genotype in rs1045642 SNP and the genotype of their renal donor. Each genotype combination has 2 sets of points along the *x* axis. The first set represents mean blood concentration of drug during the first week posttransplantation for each patient, and the second set, during the second week. When there is more than 1 patient, the median value is highlighted.

ants require lesser amounts of drug to reach the same concentration. Moreover, they could experience toxic effects if the regular dosage is too high. The effect of *CYP3A5*7* SNP could not be tested because none of the recipients exhibited this variant. Despite results in other organs, donor genotype seems to not affect drug concentration. However, further studies are needed to confirm this.

REFERENCES

1. Evans WE, Relling MV: Moving towards individualized medicine with pharmacogenomics. Nature 429:464, 2004

2. De Jonge H, Kuypers DR: Pharmacogenetics in solid organ transplantation: current status and future directions. Transplant Rev (Orlando) 22:6, 2008

3. Mendes J, Martinho O, Simoes A, et al: Genetic polymorphisms in *CYP3A5* and *MDR1* genes and their correlations with plasma levels of tacrolimus and cyclosporine in renal transplant recipients. Transplant Proc 41:840, 2009

4. Weinshilboum R, Wang L: Pharmacogenomics: bench to bedside. Nat Rev Drug Discov 3:739, 2004

5. Provenzani A, Notarbartolo M, Labbozzetta M, et al: The effect of *CYP3A5* and *ABCB1* single nucleotide polymorphisms on tacrolimus dose requirements in Caucasian liver transplant patients. Ann Transplant 14:23, 2009