Short Communication

Influence of SLCO1B3 Genetic Variations on Tacrolimus Pharmacokinetics in Renal Transplant Recipients

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Summary: The immunosuppressive drug tacrolimus requires strict therapeutic monitoring due to its narrow therapeutic index and high interindividual variability. Organic anion transporting polypeptide 1B3 (OATP1B3) is a human hepatocyte transporter involved in the hepatobiliary elimination of diverse endogenous and exogenous substances. Genetic variations within the solute carrier (SLCO) 1B3 gene that encodes OATP1B3 may contribute to interindividual differences in tacrolimus disposition. The purpose of the present study is to investigate the association between SLCO1B3 polymorphisms and tacrolimus pharmacokinetics in renal transplant recipients. We found significant correlations between two linked coding nonsynonymous polymorphisms, T334G and G699A, and mean dose-adjusted tacrolimus trough blood concentrations during the first week post-transplantation (p = 0.04) and when the target dose (10–12 ng/ml) was obtained (p = 0.01). Patients carrying the homozygous mutant haplotype had 14.3-fold higher risk (95% confidence interval: 1.43–100; p = 0.02) of having blood tacrolimus concentrations above the median level, and thus being classified as poor OATP1B3 transporters, than carriers of one or two copies of the wild-type haplotype. This study shows, for the first time, that SLCO1B3 polymorphism is associated with tacrolimus exposure in the early post-transplant period.

Keywords: tacrolimus; drug level; renal transplants; SLCO1B3; genetic polymorphisms

Introduction

Tacrolimus, a member of the calcineurin inhibitor family, is widely used in solid organ transplantation to prevent allograft rejection. It is a critical dose-drug with a narrow therapeutic index. Moreover, its pharmacokinetic characteristics may vary greatly among individuals, and daily doses must be adjusted according to its whole blood trough concentrations. Achieving therapeutic trough levels is of paramount importance during the period immediately after transplantation to prevent subsequent graft rejection.1–3 Therefore, the identification of parameters predictive of the optimal tacrolimus dosage would be a great clinical asset in the determination of adequate tacrolimus administration.

Genetically inherited differences in drug disposition and metabolism have been shown to significantly influence an individual response to therapy and have been estimated to account for 20 to 95% of the variability of drug pharmacokinetics and pharmacodynamics.4–6 As tacrolimus is metabolized by the members of the cytochrome P450 (CYP) 3A family, we and others have shown that the CYP3A5*3 genotype is associated with its
pharmacokinetics. Organic anion transporting polypeptides (OATPs) are transporter proteins that mediate intracellular uptake of many molecules in various tissues contributing to the overall drug absorption, distribution, elimination, and consequently, influencing the systemic levels of many drugs. OATP1B3 is mainly expressed on the hepatocyte sinusoidal (basolateral) membrane and transports diverse endogenous and exogenous compounds from portal blood into hepatocytes. OATP1B3 has also been identified on human tumor cells derived from brain, lung, colon, gastric, pancreatic, gallbladder and prostate. OATP1B3 is encoded by the solute carrier (SLCO) 1B3 gene. Several SLCO1B3 nucleotide sequence variations have been identified with frequencies varying between different ethnic groups. Among them, the T334G and G699A polymorphisms were recently associated with an improved survival rate in patients with prostatic cancer due to impaired testosterone transport in cancer cells featuring these mutations and with mycophenolate mofetil pharmacokinetics in renal transplant patients co-treated with tacrolimus or sirolimus. Tacrolimus has been identified as an inhibitor of OATP1B1. Two mutations in SLCO1B1 gene that encode OATP1B1, A388G and T521C, have been shown to influence tacrolimus trough blood concentrations after liver transplantation. Since OATP1B3 exhibits overlapping substrate specificities with OATP1B1, it is reasonable to assume that tacrolimus might equally be transported by OATP1B3. The purpose of the present study is to investigate whether SLCO1B3 polymorphism is associated with tacrolimus pharmacokinetics in renal transplant recipients.

Materials and Methods

Thirty-eight white Canadian renal transplant recipients were enrolled in this prospective and observational clinical study. The study group was composed of 18 males and 20 females with a mean age at the time of transplantation of 49.5 ± 13.9 years. All patients were given an immunosuppressive therapy consisting of tacrolimus, mycophenolate mofetil, and steroids. Our standard steroid regimen was characterized by 125 mg of methylprednisolone given intravenously on the day of surgery, followed by 100 mg of oral prednisolone for day 1 post-operation, reducing by 20 mg every day from day 2 to 5 to a maintenance dose of 5 mg daily thereafter. Plasma creatinine concentrations were measured 3 times a week to quantify creatinine clearance. Acute graft rejection was confirmed by kidney biopsy according to Banff criteria. Post-transplantation diabetes was defined as a new need for oral hypoglycemic agents or insulin after transplantation. Daily tacrolimus trough blood concentration (C0) (ng/ml) and tacrolimus dosage (mg/kg) were noted for the first 3 months post-transplantation. Blood concentrations of tacrolimus were determined for all patients at two time points; during the first week (between day 3 and 7) and at 3 months after transplantation. We established the distinction since tacrolimus blood levels can be influenced by co-administration of additional medication, and during the first week after transplantation no other drug known to interact with tacrolimus was administered to the patients. Furthermore, because gastrointestinal motility is seriously disturbed the first 48 h after transplantation in most patients, we determined tacrolimus trough blood concentrations between day 3 and 7 post-op to avoid this potential confounding effect. We found no correlation between clinical outcomes (creatinine clearance, acute graft rejection and post-transplant diabetes) during the first 3 months post-transplantation and the selected key SLCO1B3 polymorphisms (Table 2). Although none of the SLCO1B3 promoter or 5’UTR variants tested correlated with tacrolimus pharmacokinetics, we found significant correlations between two frequent coding nonsynonymous polymorphisms, T334G and G699A, and tacrolimus exposure in the early post-transplant period. This association remained significant after adjusting for previously reported CYP3A5 polymorphisms associated with blood tacrolimus levels in these patients. These two variants are in complete linkage disequilibrium in the white Canadian population and in other populations. Therefore all individuals carry the same genotype for both variants that are transmitted together on the same chromosome (haplotype). The homozygous mutant haplotype (334GG and 699AA) was significantly associ-

<0.05. Continuous variables were compared using unpaired Student’s t-test when continuous variables were normally distributed or with the Mann-Whitney U test otherwise. Categorical values were compared using Fisher’s exact tests. Genotypic frequencies were compared by Hardy-Weinberg expectations using the χ² test.

Results and Discussion

The full extent of SLCO1B3 nucleotide sequence diversity in the white Canadian population was reported elsewhere. Specific SLCO1B3 polymorphisms with frequencies above 5% and with the potential to affect the expression or protein structure of OATP1B3 were selected to determine their contributions to tacrolimus pharmacokinetics (Table 1). The genotypic distribution of the selected SLCO1B3 variants in the study population was in Hardy-Weinberg equilibrium.

Mean dose-adjusted tacrolimus trough blood concentrations (C0/dose weight-adjusted ratio) were determined for all patients at two time points; during the first week (between day 3 and 7) and at 3 months after transplantation. We established the distinction since tacrolimus blood levels can be influenced by co-administration of additional medication, and during the first week after transplantation no other drug known to interact with tacrolimus was administered to the patients. Furthermore, because gastrointestinal motility is seriously disturbed the first 48 h after transplantation in most patients, we determined tacrolimus trough blood concentrations between day 3 and 7 post-op to avoid this potential confounding effect. We found no correlation between clinical outcomes (creatinine clearance, acute graft rejection and post-transplant diabetes) during the first 3 months post-transplantation and the selected key SLCO1B3 polymorphisms (Table 2). Although none of the SLCO1B3 promoter or 5’UTR variants tested correlated with tacrolimus pharmacokinetics, we found significant correlations between two frequent coding nonsynonymous polymorphisms, T334G and G699A, and tacrolimus exposure in the early post-transplant period. This association remained significant after adjusting for previously reported CYP3A5 polymorphisms associated with blood tacrolimus levels in these patients. These two variants are in complete linkage disequilibrium in the white Canadian population and in other populations. Therefore all individuals carry the same genotype for both variants that are transmitted together on the same chromosome (haplotype). The homozygous mutant haplotype (334GG and 699AA) was significantly associ-

| Table 1 | Selected SLCO1B3 polymorphisms |
|-----------------|------------------------|----------------------|----------------------|
| Location       | Nucleotide substitution | Amino acid variation | Allele frequency (%) |
| Promoter       | −5838 CATCACA −5831 > del | 19.5                 |                      |
| Promoter       | −5828A > C             | 25.6                 |                      |
| Promoter       | −5538T > C             | 9.8                  |                      |
| 5’UTR          | −28 ATATACCTTGGTATCTG   | 24.4                 |                      |
| 5’UTR          | −11 > del              |                      |                      |
| 5’UTR          | −7 TTAA −4 > del       | 24.4                 |                      |
| Exon 4         | 334T > G               | Ser112Ala            | 86.6                 |
| Exon 7         | 699G > A               | Met233Ile            | 86.6                 |
| Exon 8         | 767G > C               | Gly256Ala            | 12.2                 |

UTR: untranslated region, del: deletion polymorphism. Polymorphism positions are given from the initiation translation site (ATG) or the nearest exon according the reference SLCO1B3 sequence (GenBank NC_000012 region 208553800 to 209614800).
Table 2. Pharmacokinetic parameters of tacrolimus according to SLC01B3 T334G-G699A genotypes

<table>
<thead>
<tr>
<th>T343G-G699A genotypes</th>
<th>Homozygotes 334T-699G (n = 9)</th>
<th>Homozygotes 334G-699A (n = 29)</th>
<th>p-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>log tacrolimus C0/Dose at target dose (SD) a</td>
<td>4.28 (0.47)</td>
<td>4.94 (0.63)</td>
<td>0.010</td>
</tr>
<tr>
<td>log tacrolimus C0/Dose 3–7 days (SD) a</td>
<td>4.33 (0.47)</td>
<td>4.84 (0.66)</td>
<td>0.040</td>
</tr>
<tr>
<td>log tacrolimus C0/Dose 3 months (SD) a</td>
<td>4.69 (0.36)</td>
<td>4.95 (0.57)</td>
<td>0.120</td>
</tr>
<tr>
<td>GFR at 3 months (SD) b</td>
<td>53 (15)</td>
<td>61 (14)</td>
<td>0.200</td>
</tr>
<tr>
<td>Graft rejection (%) d</td>
<td>3 (13)</td>
<td>6 (21)</td>
<td>0.430</td>
</tr>
<tr>
<td>Post-transplantation diabetes (%) e</td>
<td>3 (17)</td>
<td>9 (36)</td>
<td>0.930</td>
</tr>
</tbody>
</table>

*p-value* as determined by the Mann-Whitney U test for GFR; Student’s t-test for log C0/Dose when target dose was obtained, log C0/Dose 3–7 days and 3 months; Fisher’s test for graft rejection and post-transplantation diabetes.

aAverage of log tacrolimus C0/dose (ng/mL)/(mg/kg) when target dose (10–12 ng/mL) was obtained.

bAverage of log tacrolimus C0/dose (ng/mL)/(mg/kg) between day 3 and 7 post-transplantation.

cAverage of log tacrolimus C0/dose (ng/mL)/(mg/kg) for 3 months post-transplantation.

dAverage glomerular filtration rate in mL/min/1.73 m2 for 3 months post-transplantation.

eGraft rejection episode up to 1 year post-transplantation.

Acknowledgments: We thank Jo-Ann Fugère from the CHUM Renal Transplant Unit for providing the clinical data.

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