Racial Influence on ABCB1 Gene Expression in Peripheral Blood Mononuclear Cells in Stable Renal Transplant Recipients

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Abstract

Introduction

- Immunosuppressive agents such as cyclosporine and tacrolimus are substrates for P-glycoprotein (P-gp) efflux transporters.
- These drugs elicit their respective pharmacologic mechanism by intracellular action within the activated lymphocytes to prevent graft rejection. (See Figure 1)
- Since T lymphocytes express P-gp, alterations in P-gp expression may modify the overall pharmacologic effects from these immunosuppressive agents in the transplant recipient.
- Increased expression of ABCB1 (MDR1) in lymphocytes, which encodes for P-gp, may be associated with resistance to immunosuppression and development of graft rejection.
- In addition, African Americans have been described to have single nucleotide polymorphisms (SNPs) of ABCB1 which modulate cellular efflux of this drug. P-gp (CD25) which modulates cellular efflux of this drug. P-gp (ABCB1) gene expression was noted with greater expression in Caucasians than African Americans. These racial differences in ABCB1 gene expression may influence intracellular tacrolimus concentrations mediated by P-gp and affect clinical outcomes relative to African Americans and Caucasians.

Methods

- An observational study was completed in 20 African American (AA) and 11 Caucasian (C) stable renal transplant recipients (RTR) (ages 30-74 years) receiving tacrolimus (tacrolimus: 5-10 ng/ml), and enteric coated mycophenolate sodium. At time 0 (prior to IT) 4, 8, and 12 hours after immunosuppression, PBMCs were collected for ABCB1 gene expression analysis by quantitative real-time polymerase chain reaction (Q-PCR). The target ABCB1 gene PCR product was cloned, and verified by sequencing. The cloned gene ABCB1 was used to establish standard curves (linear over 6 orders of magnitude; r = 0.999) and assess PCR efficiencies. Total ABCB1 copies and normalized copies using Alien RNA were assessed.

Results

- The normalized (p=0.038) and non-normalized (p=0.0001) ABCB1 gene expression was higher among Caucasians and at each time until 12 hours. See Table below.

Conclusions

- The racial differences in ABCB1 gene expression was noted with greater expression in Caucasians than African Americans. These racial differences in ABCB1 gene expression may influence intracellular tacrolimus concentrations mediated by P-gp and affect clinical outcomes relative to African Americans and Caucasians.

Study Design:

- An open-label, single center observational PK-pharmacodynamic study in 20 African American and 11 Caucasian male RTR who were clinically stable receiving oral immunosuppression: tacrolimus (Prograf), enteric coated mycophenolate sodium (EC-MPS) for ~6 months was completed.

Study Day Procedure:

- Patients were admitted to the Clinical Research Center at 7AM after an overnight fast and an IV angiocatheter was inserted.
- Time zero blood samples were collected using Cell Preparation Tubes (CPT®) followed by administration of oral tacrolimus and other immunosuppressive drugs. Food and other medications were given after 2 hours.
- Blood samples for MDR1 gene expression were collected at baseline (Time 0) and 4, 8 and 12 hours post oral immunosuppression in Cell Preparation Tubes with sodium citrate (CP2007:81:2).

RNA Isolation and cDNA Synthesis:

- Total RNA was isolated from 200 uL of PBMC as per manufacturers’ protocols (Qiagen, Valencia, CA). First strand cDNA was synthesized in a 50 uL reaction containing 1 uL oligo (dT12-18 at 500 uL), 2 uL AmpliRNA control as internal standard (S1198:250 uL), and 30 uL total RNA (100 ng of total RNA), and 1 uL Stratascript™ reverse transcriptase (60:50 U/l) for reverse transcription process.

Quantitative real-time PCR:

- MRPS2 gene expression was determined using 25 uL reaction volume containing 12.5 uL RT2 SYBR Green/ROX qPCR master mix (SuperArray, California, USA), 10.5 uL ddH2O, 1 uL template cDNA, and 1 uL MRPS2 gene specific 10 uM PCR primer pair stock. The PCR reactions were initiated with denaturation at 95 °C for 10 minutes, followed 40 cycles of amplification at 95 °C for 15 seconds, and annealing at 60 °C for 60 seconds. The PCR cycles were followed by a dissociation curve analysis to confirm the single PCR product (verified by gel analysis).

- Normalization: To correct for experimental errors, samples were normalized using QRT-PCR data based upon AmpliRNA primers.

Statistical Analysis:

- The normalized (p=0.0008) and non-normalized (p=0.0001) ABCB1 gene expression in PBMCs was higher among Caucasians and at each time until 12 hours.

Conclusions

- The racial differences in ABCB1 gene expression was noted with greater expression in Caucasians than African Americans.
- These racial differences in ABCB1 gene expression may influence intracellular tacrolimus concentrations mediated by P-gp and affect clinical outcomes relative to African Americans and Caucasians.

Table 1: Patient Demographics

<table>
<thead>
<tr>
<th>Patient Demographics</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>African Americans</td>
<td>10/17</td>
</tr>
<tr>
<td>Caucasians</td>
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<td>Female</td>
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</tbody>
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Summary

- The normalized (p=0.0008) and non-normalized (p=0.0001) ABCB1 gene expression in PBMCs was higher among Caucasians and at each time until 12 hours.

Figure 1: Pharmacologic Immunosuppression

Study Objectives

- To conduct a study to quantify MDR1 (ABCB1) gene expression from peripheral blood mononuclear cells (PBMCs) prior to (trough of immunosuppressive drug) compared to 4, 8 and 12 hours after administration of immunosuppressive regimen of Tacrolimus and Mycopholic Acid in relation to race and time within the dosing interval.