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

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Abstract

Background: Immunosuppressive therapy (IT) such as tacrolimus are substrates for P-glycoprotein (P-gp) which modulates cellular efflux of this drug. P-gp is present on peripheral mononuclear cells (PBMC) and is encoded by the ABCB1 gene. No data are available regarding the impact of race on ABCB1 gene expression in PBMCs post-transplant at IT dosing interval.

Methods: An observational study was completed in 20 African American (AA) and 11 Caucasian (C) stable renal transplant recipients (RTRs) (ages 30-74 yrs) receiving tacrolimus (tacrolimus trough; 5 - 10 ng/ml), and enteric coated mycophenolate sodium. At time 0 (prior to IT) & 4, 8 and 12 hours after immunosuppressive therapy, PBMCs were collected for ABCB1 gene expression analysis by quantitative real-time polymerase chain reaction (QRT-PCR). The target ABCB1 gene PCR product was cloned, and verified by sequencing. The cloned ABCB1 gene was used to establish standard curves (linear over 6 orders of magnitude; r²=0.986) and assess PCR efficiencies. Total ABCB1 copies and normalized copies using Alien RNA were assessed.

Results: The normalized (p=0.0086) 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.

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 of ABCB1 which may reduce the pharmacologic response to immunosuppressive agents and subsequent graft survival.
• Our research group has demonstrated time dependent change in ABCB1 expression in PBMCs in relation to IL cyclosporine drug therapy (Tornatore et al., Clin. Pharmacol Ther 2007:81:2).
• However, it is unclear to date if the ABCB1 expression changes in relation to race or gender.

Methods

• Study Design: An open-label, single center observational PK/pharmacogenomic study in 20 African American and 11 Caucasian male RTRs 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 with other immunosuppressive drugs. Food and other medications were given after 2 hours.
• Blood whole blood samples were collected 4, 8, and 12 hours post-dose.
• 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 (CP2007-8D Vacutainer). Plasma was aspirated and peripheral mononuclear cells (PBMCs) were harvested immediately frozen in liquid nitrogen and stored at -70°C until Q-PCR analysis.

Patient Demographics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>African Americans</th>
<th>Caucasians (N=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46 ± 13</td>
<td>51 ± 11</td>
</tr>
<tr>
<td>Ext. GFR (ml/min)</td>
<td>37.5 ± 13.9</td>
<td>32.0 ± 19.5</td>
</tr>
<tr>
<td>Tacrolimus Trough (ng/ml)</td>
<td>7.4 ± 2.2</td>
<td>7.3 ± 1.3</td>
</tr>
<tr>
<td>Mycophenolic Acid (mg/L)</td>
<td>42.0 ± 3.3</td>
<td>58.0 ± 5.6</td>
</tr>
<tr>
<td>Metyrapone Trough (mg/L)</td>
<td>46.0 ± 31.3</td>
<td>41.4 ± 31.9</td>
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</tbody>
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Results

• The normalized (p=0.0006) 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 normalized (p=0.0006) and non-normalized (p=0.0001) ABCB1 gene expression was higher among Caucasians and at each time until 12 hours.

Statistical Analysis

• Paired samples ANOVA was utilized to evaluate differences for each time point (p≤0.05). Statistical analyses were performed using GraphPad software (ver. 6).
• The differences were assessed by the two-tailed paired Student’s t-test (p≤0.05).
• A 95% confidence interval was used to determine statistical significance.

Summary

• The normalized (p=0.0006) and non-normalized (p=0.0001) ABCB1 gene expression was higher among Caucasians and at each time until 12 hours.

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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 Mycophenolic Acid in relation to race and time within the dosing interval.