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

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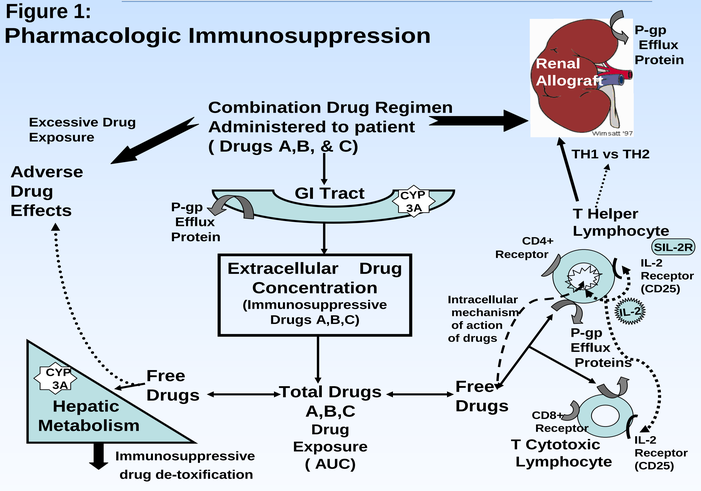
Abstract

Background: Immunosuppressive therapy (IT) such as tacrolimus are influenced by 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 *ABCB*1 gene expression in PBMCs post-transplant over IT dosing interval.
Methods: An observational study was completed in 20 African American (AA) and 11 Caucasian (C) stable renal transplant recipients (RTR) (ages 30-74 yrs) receiving tacrolimus (trough: 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 (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; r^2=0.996) and assess PCR efficiencies. Total *ABCB1* copies and normalized copies using Alien RNA were assessed.
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 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

ABCB1 Copies x 10^6 Total RNA	0 Hours	4 Hours	8 hours	12 Hours
African Americans	28.0 ± 14.6	25.0 ± 16.8	26 ± 15.2	33.4 ± 24.7
Caucasians	61.1 ± 46.3	57.2 ± 48.6	56.5 ± 47.8	53.8 ± 24.2
Post hoc Pair-wise p values	0.003	0.004	0.006	0.08

Figure 1:
Pharmacologic Immunosuppression



Study Objectives

- To conduct a study to quantitate 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.

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* compared to Caucasians 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 I 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 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 with other immunosuppressive drugs. Food and other medications were given after 2 hours.
- Whole blood samples were collected 4,8, and 12 hours post-dose.
- Blood samples for MDR-1 gene expression were collected at baseline (Time 0) and 4 , 8 and 12 hours post oral immunosuppression in Cell Preparation Tubes with sodium citrate (*CPT®-BD Vacutainer*). Plasma was aspirated and peripheral mononuclear cells (PBMCs) were harvested immediately; frozen in liquid nitrogen and stored at -70ºC until Q-PCR analysis.

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 g/uL), 2 uL Alien® RNA control as internal standard (5x108 Copies/uL), and 30 uL total RNA (100 ng of total RNA), and 1uL StrataScript™ reverse transcriptase (50 U/uL) for reverse transcription process.

Quantitative real-time PCR:
MRP2 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 ddH2O, 1 uL template cDNA, and 1 uL MRP2 gene specific 10 uM PCR primer pair stock. The PCR reactions were initiated with denaturation at 95 ºC for 10 minutes, followed 40 cycles of amplification at 95 ºC for 15 seconds, and annealing at 60 ºC for 60 seconds. The PCR cycles were followed by a dissociation curve analysis to confirm the single PCR product (verified by gel analysis).

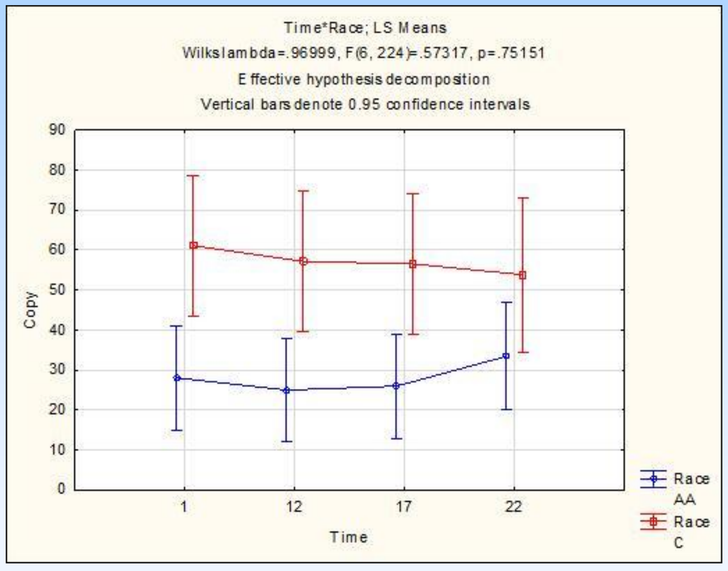
QRT-PCR standard curves and target verification:
The PCR product was cloned into a plasmid vector (pCR2.1-TOPO) using the TOPO™ TA Cloning kit and sequenced to verify as MRP2. Cloned PCR product was utilized to establish standard curves of known concentrations for absolute quantitation of MRP2 gene expression levels and to assess efficiency of each PCR run. Each sample was analyzed in triplicate.
Normalization:
To correct for experimental errors, samples were normalized using QRT-PCR data based upon Alien® primers.

Statistical Analysis:
Repeated measures ANOVA was utilized to evaluate differences as follows:
- Timed differences between race at time 0 hours (trough)
- Timed differences between race at times 4, 8 and 12 hours
- ANOVA was used to determine difference between Caucasian female and male patients

Results

Patient Demographics

Demographics Mean ± SD	African Americans (N=20)	Caucasians (N=11)	P Value (NS: Non-significant)
AGE (years)	48 ± 13	51 ± 11	NS
Est. GFR (ml/min)	57.5 ± 13.9	62.6 ± 19.5	NS
Tacrolimus Trough (ng/ml)	7.4 ± 2.2	7.3 ± 1.3	NS
Mycophenolic Acid Trough (mg/dL)	4.2 ± 3.3	5.8 ± 5.6	NS
Mycophenolate Glucuronate (mg/dL)	46.0 ± 31.9	41.4 ± 31.9	NS
Time Post-Transplant (Mos)	28.9 ± 21.5	58.8 ± 53.8	NS
Diabetes	30%	10%	NS



Summary

- The normalized (p<0.0006) 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