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Functional consequences of pravastatin isomerization on OATP1B1-mediated transport

Jonathan Wagner[1], Melissa Ruggiero, J. Steven Leeder and Bruno Hagenbuch
[1]University of Kansas Frontiers

OBJECTIVES/SPECIFIC AIMS: In the present study, we examined the functional consequences of 3α-PVA on OATP1B1-mediated PVA transport. To elucidate this, we determined the effect of SLCO1B1 genotype on PVA transport and the role of 3αPVA as a competitive inhibitor of OATP1B1, which could serve as another covariate that disrupts the systemic and hepatic exposure of pravastatin in children and adults. METHODS/STUDY POPULATION: Site directed mutagenesis was performed to generate SLCO1B1 genotypes of interest (*1a, *1b, *5, *15). Human embryonic kidney (HEK293) cells were grown and plated at 200 000 cells per well in 24-well plates. Twenty-four hours later the cells were transfected with the aforementioned plasmids. Forty-eight hours later cell-based transport was performed with radiolabelled [3H]-pravastatin sodium salt. Non-radioactive pravastatin sodium salt and 3'α-iso-pravastatin sodium salt was used for PVA transport and 3αPVA studies, respectively. Cells were washed 3 times with warm uptake buffer, incubated for 1 minute with uptake solutions containing PVA and 3αPVA at varying concentrations. Transport was terminated by four 1-ml washes with ice-cold uptake buffer. Cells were lysed with 300 µl 1% Triton X-100 in PBS at room temperature for 30 minutes prior to analysis. Radioactivity was measured in a MicroBeta2 liquid scintillation counter. The remaining cell lysates were transferred to 96-well plates to determine total protein concentration using the bicinchonic acid protein assay. All transport measurements were corrected by the total protein concentration. All experiments were performed 3 to 4 times independently with 2-3 determinations. Data were analyzed for significant differences amongst genotype groups using ANOVA followed by Tukey's multiple comparisons test. IC50 and kinetic parameters were calculated using non-linear regression analysis. RESULTS/ANTICIPATED RESULTS: Pravastatin transport in SLCO1B1 variants (*5, *15) was significantly decreased compared to the reference genotype *1a and *1b (Km [µM]: *1a 18.2 ± 0.9; *1b 17.9 ± 3.3; *5 34.2 ± 9.7; *15 34.1 ± 6.1; $p \leq 0.05$; Vmax [pmol/mg/min]: *1a 104.9 ± 13.1; *1b 93.7 ± 16.7; *5 44.8 ± 15.9; *15 62.3 ± 22.5; $p \leq 0.05$). *1a and *1b were not significantly different with respect to pravastatin transport. Intrinsic clearance was diminished nearly 4 to 5-fold in SLCO1B1 variants compared to reference genotypes (Vmax/Km [µl/min/mg]: *1a 5.8 ± 0.8; *1b 5.7 ± 1.9; *5 1.3 ± 0.2; *15 1.8 ± 0.3; $p \leq 0.01$). Pravastatin transport was inhibited by 3αPVA for all genotypes. However, there was more pronounced inhibition in the SLCO1B1 variant genotypes compared to reference genotypes (IC50 [µM]: *1a 15.9 ± 1.9; *1b 18.6 ± 5.7; *5 3.9 ± 2.0; *15 4.4 ± 0.8; $p \leq 0.01$; Ki: *1a 15.0 ± 1.8; *1b 17.5 ± 5.4; *5 3.8 ± 2.9; *15 4.3 ± 0.8; $p \leq 0.01$). DISCUSSION/SIGNIFICANCE OF IMPACT: In vitro PVA transport is altered according to SLCO1B1 genotype, consistent with previous in vitro and human experience. Our data suggest that the significantly different maximal transport velocity (Vmax) in variant versus non-variant genotypes is consistent with decreased membrane expression of OATP1B1 with the variant c.521T>C allele. However, in contrast to data involving typical model substrates (e.g. estrone-3-sulfate), the PVA binding affinity (Km) was significantly different between variant and non-variant genotypes, consistent with altered binding of the substrate to OATP1B1. Collectively, we conclude that decreased OATP1B1 expression and function in variant genotypes influence altered transport for PVA. Finally, the functional consequences of 3αPVA formation on PVA transport was confirmed in our study. Mechanistically, we confirmed our observation in humans that 3αPVA inhibits OATP1B1 transport. However, this effect is more pronounced in variant genotypes as shown by lower IC50 values compared to the reference genotypes. This highlights another source of variation that must be taken into consideration when trying to optimize the pravastatin dose-exposure relationship in humans.

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Gendered racism, psychological distress, and the strong Black woman

Maha Baalbaki
Marquette University

OBJECTIVES/SPECIFIC AIMS: Black women experience discrimination that targets their intersecting gender and ethnic identities, termed gendered racism (Essed, 1991). The gendered racism Black women experience has been linked to negative mental health outcomes (Thomas et al., 2008). The 'strong Black woman' is a cultural symbol of strength depicting the Black woman as unwavered by hardships, such as gendered racism (Shorter-Gooden & Washington, 1996). However, recent research suggests that belief in the strong Black woman cultural construct is associated with negative mental health outcomes (Watson & Hunter, 2015). The goals of the current study were to (1) replicate previous findings suggesting that experiences with gendered racism is positively correlated with psychological distress, (2) replicate previous findings suggesting that belief in the strong Black woman construct is positively correlated with psychological distress, and (3) explore how experiences with gendered racism and belief in the strong Black woman construct might interact to predict distress. METHODS/STUDY POPULATION: A national sample of 112 Black women completed an online survey via MTurk. Survey measures included the Gendered Racial Microaggressions Scale, Strong Black Woman Cultural Construct Scale, and Psychological Distress Scale. RESULTS/ANTICIPATED RESULTS: Pearson correlation revealed that experiences with gendered racism was positively correlated with psychological distress, $r = 0.23$, $p = .02$. Pearson correlation also revealed that belief in the strong Black woman cultural construct was positively correlated with psychological distress, $r = 0.39$, $p < .001$. Multiple linear regression revealed an interaction between experiences with gendered racism and belief in the strong Black woman construct ($\beta = -0.18$, $p = .04$) that predicted psychological distress, $R2 = .20$, $F(3,108) = 8.63$, $p < .01$. Namely, for those with high belief in the strong Black woman construct, experiences with gendered racism did not predict distress, $\beta = -0.31$, $t = -0.29$, $p = .78$. However, for those with low belief in the construct, experiences with gendered racism positively predicted distress, $\beta = -2.57$, $t = 2.31$, $p = .02$. DISCUSSION/SIGNIFICANCE OF IMPACT: The results underscore the harmful effects of gendered racism and gendered racial stereotypes on Black women's mental health outcomes. Striving to appear as the strong Black woman is not likely to help Black women overcome daily hardships. In fact, belief in the strong Black woman construct is likely to add extra difficulties.

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