Pharmacokinetic Changes during Pregnancy According to Genetic Variants: a Prospective Study in HIV-Infected Patients Receiving Atazanavir-Ritonavir

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ABSTRACT Atazanavir-ritonavir concentrations change over time during pregnancy in HIV-positive patients; the impact of genetic variants is unknown. Twenty patients were enrolled in this study; plasma and intracellular concentrations of antiretrovirals were measured, in addition to single-nucleotide polymorphisms in transport-related genes. Linear logistic regression showed that genetic variants in organic-anion-transporter-1B1- and pregnane-X-receptor-encoding genes affected third-trimester atazanavir exposure. In this prospective study, genetic variants partially explained the observed interpatient variability in third-trimester exposure to antiretrovirals.

KEYWORDS HIV, antiretroviral agents, antiviral pharmacology

Antiretroviral (ARV) treatment of HIV-infected pregnant women is a key element to preventing mother-to-child HIV transmission (MTCT) (1). Despite the target of ARVs being intracellular, knowledge of the pharmacokinetics of these compounds mostly derives from plasma concentration. Some reports suggested a potential correlation between the intracellular (IC) protease inhibitor (PI) concentrations, their activities, and clinical outcomes (2–7). One of the most preferred classes of anchor drugs of ARV regimen during pregnancy are protease inhibitors (PIs); however, several changes in PI exposure have been reported in the third trimester (10). Following oral administration, this class of drugs is primarily metabolized by cytochrome P450 (CYP) 3A4 and is a substrate of the transmembrane efflux pump P-glycoprotein (P-gp) and of the organic anion transporters protein 1B1 (OATP1B1) (8, 9).

All of these steps may be mediated by proteins regulated by different genes. Single-nucleotide polymorphisms (SNPs) in pregnane-X-receptor (PXR), P-gp, and OATP1B1 have been involved in intracellular drug penetration (10, 11). PXR is a nuclear transcription factor that influences the expression of P-glycoprotein (26); PXR regulates the expression of CYP3A4 and ABCB1 and is associated with atazanavir plasma concentration (T allele at position 63396) (12, 13). Several studies have reported a relationship between the ABCB1 3435 C/T polymorphism and PI activity (14, 15). SNPs in the OATP1B1-encoding gene, in particular the 521C allele, have been associated with a decrease in PI transport, both *in vitro* and *in vivo* (16–19). Pregnancy induces changes in systemic gene expression, but there are a lack of data about transporting genes changes during pregnancy (20).

The aim of this study was to evaluate changes in atazanavir (ATV) and ritonavir (RTV) plasma/IC concentrations during pregnancy according to SNPs in PXR-, P-gp-, and OATP1B1-encoding genes.

HIV-positive pregnant patients treated with an ATV-RTV combination (300/100 mg

Received 22 February 2018 Returned for modification 15 March 2018 Accepted 21 April 2018

Accepted manuscript posted online 14 May 2018

Citation Focà E, Calcagno A, Bonito A, Cusato J, Domenighini E, D'Avolio A, Quiros Roldan E, Trentini L, Castelnuevo F, Di Perri G, Castelli F, Bonora S. 2018. Pharmacokinetic changes during pregnancy according to genetic variants: a prospective study in HIV-infected patients receiving atazanavir-ritonavir. *Antimicrob Agents Chemother* 62:e00309-18. <https://doi.org/10.1128/AAC.00309-18>.

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TABLE 1 Patient characteristics

Variable ^a	Data
Age (median [IQR]) (yr)	33.8 (28.2–36.4)
Caucasian ethnicity (no. [%])	13 (65)
BMI (median [IQR]) (kg/m ²)	24.7 (23–32.5)
Previous AIDS-defining events (no. [%])	2 (10)
HBsAg positivity (no. [%])	2 (10)
HCV-Ab positivity (no. [%])	1 (5)
ARV treatment backbone (no. [%])	
TDF-FTC	17 (85)
ABC-3TC	3 (15)

^aIQR, interquartile range; BMI, body mass index; HBsAg, hepatitis B surface antigen; HCV-Ab, hepatitis C virus antibody; ARV, antiretroviral; TDF-FTC, tenofovir-emtricitabine; ABC-3TC, abacavir-lamivudine.

once daily, with food)-based regimens were prospectively enrolled after signing a written informed consent.

Plasma and IC (intrapерipheral blood mononuclear cell [intra-PBMC]) antiviral concentrations of ATV and RTV were measured at every visit using validated high-performance liquid chromatography-mass spectrometry (HPLC-MS) methods (with direct evaluation of cell volume) (21, 22).

PBMC-associated and plasma ATV and RTV concentrations were measured by validated HPLC-MS and HPLC-photodiode array (HPLC-PDA) methods, respectively. Median values of individual measurements were considered and expressed as nanograms per milliliter.

Whole-blood samples were stored at –20°C for pharmacogenetic analysis. Genomic DNA was extracted using a QIAamp whole-blood minikit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. Genotyping was conducted by real time-based allelic discrimination with the use of standard methods (Applied Biosystems, Foster City, CA, USA). All primers, probes, and PCR conditions used are available on request. The single-nucleotide polymorphisms analyzed were *PXR* 63396 C→T (rs2472677, encoding *PXR*), *ABCB1* 3435 C→T (rs1045642, encoding P-glycoprotein), and *SLCO1B1* 521 T→C (rs4149056, encoding OATP1B1) and were grouped as dichotomous variables (according to available literature data).

For descriptive statistics, continuous variables were described as medians (25th to 75th percentiles [interquartile range {IQR}]) and categorical variables as frequencies (percentages). All polymorphisms were tested for Hardy-Weinberg equilibrium (<http://www.oege.org/software/hwe-mr-calc.shtml>). Mann-Whitney analysis was used to compare previously identified relevant genotypes (*PXR* 63396TT, *ABCB1* 3435CT/TT, and *SLCO1B1* 521TC/CC) with plasma and IC concentrations, as well as IC-to-plasma ratios (IPr) and changes over time. Linear regression analysis was used to investigate the potential influences of the three SNPs on plasma and IC concentrations changes over time. Statistical analyses were performed using the PASW software package version 23.0 (SPSS, Chicago, IL, USA), accepting statistical significance at two-sided *P* values of <0.05.

Twenty HIV-positive pregnant women were enrolled from November 2013 to January 2016. Baseline characteristics are reported in Table 1. The viroimmunological, clinical, and pharmacokinetic results have been reported elsewhere (23).

PXR 63396 CC, CT, and TT genotypes were observed in 6 (30%), 8 (40%), and 6 (30%) individuals, respectively; *ABCB1* 3435 CC, CT, and TT genotypes were observed in 12 (60%), 5 (25%), and 3 (15%) individuals, respectively; and *SLCO1B1* 521 TT, TC, and CC genotypes were observed in 16 (80%), 3 (15%), and 1 (5%) individuals, respectively. All SNPs were in Hardy-Weinberg equilibrium (*P* > 0.05). In *PXR* 63396 TT carriers, third-trimester ATV concentrations were lower in plasma (468 ng/ml [range, 262 to 746 ng/ml] versus 810 ng/ml [range, 341 to 1,338 ng/ml], respectively; *P* = 0.39) and higher intracellularly (1,224 ng/ml [range, 823 to 1,967 ng/ml] versus 640 ng/ml [range, 394 to 1,906 ng/ml], respectively; *P* = 0.11); IPr were significantly higher (2.6 [range, 1.6 to 3.5] versus 0.96 [range, 0.5 to 2]; *P* = 0.03, respectively).

FIG 1 Intracellular-to-plasma ratios of atazanavir (above) and ritonavir (below) according to single nucleotide polymorphisms in the studied genes. Boxes (ordered according to trimester of pregnancy) represent interquartile ranges, horizontal lines represent median values, and whiskers represent standard deviations; circles and stars represent outliers.

Compared to postpartum, ATV plasma concentrations were not significantly lower (negative values indicate decreases and positive values indicate increases) in the third trimester (−315 ng/ml [−1,121 to +129 ng/ml]), with stable IPr (+0.12 ng/ml [−0.44 to +1.35 ng/ml]). PXR 63396 TT carriers, despite a greater decrease in plasma concentrations (−397 versus −247 ng/ml, respectively), showed an increase in IC levels (+95 versus −241 ng/ml, respectively) and in IPr (+0.42 versus +0.12, respectively). The association between ATV-RTV plasma concentrations, IC concentrations, and IPr according to the studied SNPs are shown in Fig. 1 and in Table S1 in the supplemental material.

Logistic regression analysis (including all three genotypes) identifying predictors of third-trimester changes (versus postpartum concentrations) showed the *SLCO1B1* 521 TC/CC genotypes to be predictors of ATV changes ($P = 0.059$ for plasma and $P = 0.051$ for IC) and PXR 63396 TT genotypes to be predictors of RTV changes (IPr, $P = 0.025$) in the third trimester.

We observed minimal changes in ATV and RTV concentrations in HIV-positive pregnant patients; this variability could be explained by genetic variants (23).

For this purpose, we studied SNPs in genes encoding OATP1B1, P-gp, and PXR. PXR may influence the expression of P-gp as well as other membrane transporters, and it is activated by a huge variety of endobiotics and xenobiotics, including several drugs (24, 25). It has been demonstrated that PXR regulates the expression of CYP3A4/5 and P-gp-encoding genes and it has been associated with ATV plasma exposure (T allele at position 63396) (12, 13). We observed that a TT genotype in PXR 63396 was associated with higher ATV IPr and less variability in IC concentrations during the third trimester. Furthermore, multivariate logistic regression analysis identified single-nucleotide polymorphisms in OATP1B1-encoding genes as being relevant for ATV plasma concentration changes during the third trimester. Available data are limited but suggest that hepatic uptake (and therefore the amount of drug available for metabolism) may be mediated by OATP1B1 transport (8).

Possible limitations of this study are the limited sample size, the limited amount of drug measurement, and the lack of serum markers that could help in understanding the complex interplay between genetically determined and pregnancy-induced changes.

Further studies may clarify the role of different SNPs on antiretroviral transport during pregnancy, as well as the clinical impact of IC ATV and RTV accumulation according to different genotypes. These results may be relevant for allowing tailored treatment strategies during pregnancy.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00309-18>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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