Pharmacokinetic Changes during Pregnancy According to Genetic Variants: A Prospective Study in HIV-infected Patients Receiving Atazanavir/Ritonavir

Emanuele Focà¹*, Andrea Calcagno²*, Andrea Bonito¹, Jessica Cusato²,
Elisabetta Domenighini¹, Antonio D’Avolio², Eugenia Quiros Roldan¹, Laura Trentini²,
Filippo Castelnuovo¹, Giovanni Di Perri², Francesco Castelli¹, Stefano Bonora²

¹Unit of Infectious and Tropical Diseases, Department of Clinical and Experimental Sciences, University of Brescia and Spedali Civili General Hospital, Brescia, Italy;
²Unit of Infectious Diseases, Department of Medical Sciences, University of Torino, Torino, Italy

* These Authors equally contributed to this work

Corresponding Author
Emanuele Focà, MD, PhD
Unit of Infectious and Tropical Diseases, Department of Clinical and Experimental Sciences
University of Brescia, Piazzale Spedali Civili 1, 25136 Brescia
Emanuele.foca@unibs.it
Abstract

Atazanavir/ritonavir concentrations change over pregnancy in HIV-positive patients; the impact of genetic variants is unknown. 20 patients were enrolled: antiretrovirals’ plasma and intracellular concentrations were measured in addition to single nucleotide polymorphisms in transport affecting 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 inter-patient variability in third-trimester antiretrovirals’ exposure.
Main text

Antiretroviral (ARV) treatment of HIV-infected pregnant women is a key element to prevent mother-to-child HIV transmission (MTCT) [1]. Despite ARVs’ target being intracellular the knowledge on these compounds pharmacokinetics mostly derives from plasma concentration. Some reports suggested a potential correlation between PIs’ ICs, their activity and clinical outcomes [2, 3, 4, 5, 6, 7]. One of the most preferred third agents of ARV regimen during pregnancy are protease Inhibitors (PIs); however several changes in PIs exposure have been reported in third trimester. Following oral administration this class of drugs is primarily metabolized by cytochrome P450 (CYP) 3A4 and is 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 [11, 12]. PXR is a nuclear transcription factor that influences the expression of P-glycoprotein; PXR regulates the expression of CYP3A4 and ABCB1 and is associated with atazanavir plasma concentration (T allele at position 63396) [13,14]. Several studies have reported a relationship between the ABCB1 3435 C/T polymorphism and PIs activity. [15, 16] SNPs in OATP1B1 encoding gene, in particular the 521C allele, have been associated with a decrease in PIs transport, both in vitro and in vivo. [17,18,19,20]. Pregnancy induces changes in systemic gene expression but there is lack of data about transporting genes changes during pregnancy [21].

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

HIV- pregnant patients treated with ATV/RTV (300/100 mg once daily, with food) based regimens were prospectively enrolled after signing a written informed consent.

Plasma and IC (intra-peripheral blood mononuclear cell, PBMC) antiviral concentration of ATV and RTV were measured at every visit using validated high-performance liquid chromatography (HPLC)/mass spectrometry methods (with direct evaluation of cells volume) [22, 23].

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

Whole blood was stored at -20°C for pharmacogenetic analysis. Genomic DNA was extracted using QIAamp whole blood mini kit (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 are available on request. The following single nucleotide polymorphisms were analysed: PXR 63396 C>T (rs2472677, encoding for PXR), ABCB1 3435 C>T (rs1045642, encoding for P-glycoprotein), SLCO1B1 521 T>C (rs4149056, encoding for OATP1B1) and grouped as dichotomous variables (according to available literature data).

For descriptive statistics, continuous variables were described as medians (25th–75th percentiles, 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 ver. 23.0 (SPSS, Chicago, IL, USA), accepting statistical significance at two-sided p-values <0.05.

Twenty HIV-positive pregnant women were enrolled from November 2013 to January 2016. Baseline characteristics are reported in Table 1. Viro-immunological, clinical and pharmacokinetic results have been reported elsewhere [24].

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%) and SLCO1B1 521 TT, TC and CC genotypes were observed in 16 (80%), 3 (15%) and 1 (5%). 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 262-746) vs. 810 ng/mL (341-1338), p=0.39] and higher intracellularly [1224 ng/mL (823-1967) vs. 640 ng/mL (394-1906), p=0.11]; IPr were significantly higher [2.6 (1.6-3.5) vs. 0.96 (0.5-2), p=0.03].

As compared to post-partum, ATV plasma concentrations were not significantly lower in the third trimester [-315 ng/mL (-1121; +129)] with stable IPr [+0.12 (-0.44; +1.35)]. PXR 63396 TT carriers, despite a higher decrease in plasma concentrations (-397 vs. -247 ng/mL) showed an increase in IC levels (+95 vs. -241 ng/mL) and in IPr (+0.42 vs. +0.12). The association between ATV/RTV plasma, IC and IPr according to the studied SNPs are shown in Figure 1 and Supplementary Table 1.

Logistic regression analysis (including all three genotypes) identifying predictors of third trimester changes (versus post-partum concentrations) showed SLCO1B1 521 TC/CC genotypes as predictors.
of ATV changes (p=0.059 for plasma and p=0.051 for IC) and PXR 63396 TT genotypes 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 [24].

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 [25, 26]. 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) [14, 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 concentrations 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 understanding the complex interplay between genetically determined and pregnancy-induced changes. Further studies may clarify the role of different SNP 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.
References


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**Figure 1 Legend**

**Figure 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 median values and whiskers standard deviations; circles and stars represent outliers.