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

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

Emanuele Focà[1]*, Andrea Calcagno[2]*, Andrea Bonito[1], Jessica Cusato[2], Elisabetta Domenighini[1], Antonio D'Avolio[2], Eugenia Quiros Roldan[1], Laura Trentini[2], Filippo Castelnuovo[1], Giovanni Di Perri[2], Francesco Castelli[1], Stefano Bonora[2]

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

[2]Unit 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 monuclear cell, PBMC) antiviral concentration of ATV and RTV were measured at every visit using validated high-performance liquid chromatography (HPLC)/mass (MS) 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-immulogical, 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 hanges 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

1. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. Geneva, Switzerland: WHO, 2013.
2. Ford J, Khoo SH, Back DJ. The intracellular pharmacology of antiretro viral protease inhibitors. J Antimicrob Chemother 2004; 54: 982–90.
3. Bazzoli C, Jullien V, Le Tiec C, Rey E, Mentré F, Taburet AM.Intracellular Pharmacokinetics of Antiretroviral Drugs in HIV-Infected Patients, and their Correlation with Drug Action. Clin Pharmacokinet. 2010;49(1):17-45.
4. Khoo SH, Hoggard PG, Williams I, Meaden ER, Newton P, Wilkins EG, Smith A, Tjia JF, Lloyd J, Jones K, Beeching N, Carey P, Peters B, Back DJ. Intracellular accumulation of human immunodeficiency virus protease inhibitors. Antimicrob Agents Chemother. 2002 Oct;46(10):3228-35.
5. Crommentuyn KM, Mulder JW, Mairuhu AT, van Gorp EC, Meenhorst PL, Huitema AD, Beijnen JH. The plasma and intracellular steady-state pharmacokinetics of lopinavir/ritonavir in HIV-1-infected patients. Antivir Ther. 2004 Oct;9(5):779-85.
6. Breilh D, Pellegrin I, Rouzés A, Berthoin K, Xuereb F, Budzinski H, Munck M, Fleury HJ, Saux MC, Pellegrin JL. Virological, intracellular and plasma pharmacological parameters predicting response to lopinavir/ritonavir (KALEPHAR study). AIDS. 2004 Jun 18;18(9):1305-10.
7. Ford J, Boffito M, Maitland D, Hill A, Back D, Khoo S, Nelson M, Moyle G, Gazzard B, Pozniak A. Influence of atazanavir 200 mg on the intracellular and plasma pharmacokinetics of saquinavir and ritonavir 1600/100 mg administered once daily in HIV-infected patients. J Antimicrob Chemother. 2006 Nov;58(5):1009-16.
8. Hartkoorn RC, Kwan WS, Shallcross V, Chaikan A, Liptrott N, Egan D, Sora ES, James CE, Gibbons S, Bray PG, Back DJ, Khoo SH, Owen A. HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. Pharmacogenet Genomics. 2010 Feb;20(2):112-20.
9. Huang L, Wring SA, Woolley JL, Brouwer KR, Serabjit-Singh C, Polli JW. Induction of P-glycoprotein and cytochrome P450 3A by HIV protease inhibitors. Drug Metab Dispos. 2001 May;29(5):754-60.
10. Pavek P. Pregnane X Receptor (PXR)-Mediated Gene Repression and Cross-Talk of PXR with Other Nuclear Receptors via Coactivator Interactions. Front Pharmacol. 2016 Nov 25;7:456.

11. Focà E, Ripamonti D, Motta D, Torti C. Unboosted atazanavir for treatment of HIV infection: rationale and recommendations for use. Drugs. 2012 Jun 18;72(9):1161-73.
12. D'Avolio A, Carcieri C, Cusato J, Simiele M, Calcagno A, Allegra S, Sciandra M, Trentini L, Di Perri G, Bonora S. Intracellular accumulation of atazanavir/ritonavir according to plasma concentrations and OATP1B1, ABCB1 and PXR genetic polymorphisms. J Antimicrob Chemother. 2014 Nov;69(11):3061-6.
13. Siccardi M, D'Avolio A, Baietto L, Gibbons S, Sciandra M, Colucci D, Bonora S, Khoo S, Back DJ, Di Perri G, Owen A. Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C/T) with reduced concentrations of unboosted atazanavir. Clin Infect Dis. 2008 Nov 1;47(9):1222-5.
14. Schipani A, Siccardi M, D'Avolio A, Baietto L, Simiele M, Bonora S, Rodríguez Novoa S, Cuenca L, Soriano V, Chierakul N, Saguenwong N, Chuchuttaworn C, Hoskins JM, Dvorak AM, McLeod HL, Davies G, Khoo S, Back DJ, Di Perri G, Owen A. Population pharmacokinetic modeling of the association between 63396C->T pregnane X receptor polymorphism and unboosted atazanavir clearance. Antimicrob Agents Chemother. 2010 Dec;54(12):5242-50.
15. Eichelbaum M, Fromm MF, Schwab M. Clinical aspects of the MDR1 (ABCB1) gene polymorphism. Ther Drug Monit. 2004 Apr;26(2):180-5.
16. Michaud V, Bar-Magen T, Turgeon J, Flockhart D, Desta Z, Wainberg MA. The dual role of pharmacogenetics in HIV treatment: mutations and polymorphisms regulating antiretroviral drug resistance and disposition. Pharmacol Rev. 2012 Jul;64(3):803-33.
17. Tirona RG, Leake BF, Merino G, Kim RB. Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. J Biol Chem. 2001 Sep 21;276(38):35669-75.
18. Kohlrausch FB, de Cássia Estrela R, Barroso PF, Suarez-Kurtz G. The impact of SLCO1B1 polymorphisms on the plasma concentration of lopinavir and ritonavir in HIV-infected men. Br J Clin Pharmacol. 2010 Jan;69(1):95-8.
19. Iwai M, Suzuki H, Ieiri I, Otsubo K, Sugiyama Y. Functional analysis of single nucleotide polymorphisms of hepatic organic anion transporter OATP1B1 (OATP-C). Pharmacogenetics. 2004 Nov;14(11):749-57.
20. van der Deure WM, Friesema EC, de Jong FJ, de Rijke YB, de Jong FH, Uitterlinden AG, Breteler MM, Peeters RP, Visser TJ. Organic anion transporter 1B1: an important factor in

hepatic thyroid hormone and estrogen transport and metabolism. Endocrinology. 2008 Sep;149(9):4695-701.

21. Mittal A, Pachter L, Nelson JL, Kjærgaard H, Smed MK, Gildengorin VL, Zoffmann V, Hetland ML, Jewell NP, Olsen J, Jawaheer D. Pregnancy-Induced Changes in Systemic Gene Expression among Healthy Women and Women with Rheumatoid Arthritis. PLoS One. 2015 Dec 18;10(12):e0145204.
22. Simiele M, D'Avolio A, Baietto L, Siccardi M, Sciandra M, Agati S, Cusato J, Bonora S, Di Perri G. Evaluation of the mean corpuscular volume of peripheral blood mononuclear cells of HIV patients by a coulter counter to determine intracellular drug concentrations. Antimicrob Agents Chemother. 2011 Jun;55(6):2976-8.
23. Colombo S, Buclin T, Cavassini M, Décosterd LA, Telenti A, Biollaz J, Csajka C. Population pharmacokinetics of atazanavir in patients with human immunodeficiency virus infection. Antimicrob Agents Chemother. 2006 Nov;50(11):3801-8.
24. Focà E, Calcagno A, Bonito A, Simiele M, Domenighini E, D'Avolio A, Quiros Roldan E, Trentini L, Casari S, Di Perri G, Castelli F, Bonora S. Atazanavir intracellular concentrations remain stable during pregnancy in HIV-infected patients. J Antimicrob Chemother. 2017 Nov 1;72(11):3163-3166.
25. Chang TK, Waxman DJ. Synthetic drugs and natural products as modulators of constitutive androstane receptor (CAR) and pregnane X receptor (PXR). Drug Metab Rev. 2006;38(1-2):51-73.
26. Chen S, Yueh MF, Evans RM, Tukey RH. Pregnane-x-receptor controls hepatic glucuronidation during pregnancy and neonatal development in humanized UGT1 mice. Hepatology. 2012 Aug;56(2):658-67.

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.