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Short Communication
Atazanavir-Based Therapy Is Associated with Higher Hepatitis C Viral Load in HIV Type 1-Infected Subjects with Untreated Hepatitis C

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Abstract

We assessed the relationship between atazanavir (ATV)-based antiretroviral treatment (ART) and plasma hepatitis C virus (HCV) viral load in a population of HIV/HCV-coinfected patients. HIV/HCV-coinfected patients who received ART based on a protease inhibitor (PI) or nonnucleoside reverse transcriptase inhibitor (NNRTI) were included. Patients were stratified by ART drug [ATV/rtv, lopinavir (LPV/rtv), efavirenz (EFV), nevirapine (NVP), and other PIs], HCV genotype (1/4 and 2/3), and IL28B genotype (CC and non-CC). The Kruskal–Wallis test and chi-squared test were used to compare continuous and categorical variables, respectively. Multivariate analysis consisted of a stepwise linear regression analysis. Six hundred and forty-nine HIV/HCV-coinfected patients were included. HCV genotype 1/4 patients who received ATV had higher HCV RNA levels [6.57 (5.9–6.8) log IU/ml] than those who received LPV [6.1 (5.5–6.5) log IU/ml], EFV [6.1 (5.6–6.4) log IU/ml], NVP [5.8 (5.5–5.9) log IU/ml], or other PIs [6.1 (5.7–6.4) log IU/ml] ($p = 0.014$). This association held for the IL28B genotype (CC versus non-CC). The association was not found in patients carrying HCV genotypes 2/3. The linear regression model identified the IL28B genotype and ATV use as independent factors associated with HCV RNA levels. ATV-based therapy may be associated with a higher HCV RNA viral load in HIV/HCV-coinfected patients.

Hemoglobin catabolism is closely related to the replication of the hepatitis C virus (HCV).[1] Heme oxygenase-1 (HO-1) catalyzes the breakdown of the heme molecule to yield equimolar quantities of biliverdin (BV), iron, and carbon monoxide. The oxidation of heme by HO-1 releases at least two antiviral agents, iron and BV.[2] Lehman *et al*. reported that BV has antiviral activity in replicon cells, noting that antiviral activity was accompanied by a rise in specific interferon-stimulated gene (ISG) products.[2] Likewise, Zhu *et al*. recently demonstrated that BV has potent antiviral activity against HCV, with inhibitory action over HCV NS3/4A protease.[3] Iron has also been shown to inhibit HCV replication by preventing divalent caption binding to RdRp.[4]

Atazanavir (ATV) is a protease inhibitor used in HIV therapy. ATV interferes with hemoglobin catabolism because of its competing inhibitory activity against the uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) enzyme, responsible for bilirubin (BR) conjugation.[5] This inhibition is directly

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associated with hyperbilirubinemia, the most common side effect of patients treated with ATV boosted with ritonavir (ATV/rtv). We hypothesize that this inhibition could modify the modulatory effect of the products of hemoglobin catabolism on HCV replication.

The aim of this study therefore was to assess the relationship between the use of ATV-based antiretroviral treatment (ART) and plasma HCV viral load in a population of HIV/HCV-coinfected patients.

The study population was 829 HCV treatment-naive patients who had been consecutively enrolled from October 2004 to December 2011 in the Spanish HEPAVIR prospective cohort of HIV/HCV-coinfected patients. Further details of these cohorts have been reported elsewhere.[6] Patients who received ART based on two ITIAN plus a protease inhibitor (PI) or nonnucleoside reverse transcriptase inhibitors (NNRTI) were included in the study. Patients who received estavudine, didanosine, or zidovudine were excluded from the study. Only patients maintained for a minimum of time of 12 months on an unchanged regimen were included. HCV RNA levels in plasma were measured using a commercial PCR assay (Cobas Taqman; Roche Diagnostic Systems Inc., Pleasanton, CA: detection limit of 50 IU/ml).

Liver fibrosis stage was determined by biopsy or liver transient elastography (FibroScan, Echosen, Paris). Significant fibrosis was defined as a METAVIR fibrosis score of F3–F4 in liver biopsy or a liver stiffness (LS) value of ≥ 8.9. SNP rs129679860, located 3 kilobases upstream of the IL28B gene, was genotyped using a custom Taqman assay (Applied Biosystems, Foster City, CA) on DNA isolated from whole blood samples. The bilirubin data of those patients on ATV/rtv treatment were also collected. Patients were stratified by ART drug [ATV/rtv, lopinavir (LPV), efavirenz (EFV), nevirapine (NVP), or other PI (saquinavir, nelfinavir, darunavir, or indinavir)], HCV genotype (1/4 and 2/3), and IL28B genotype (CC and non-CC). The Kruskal–Wallis test and chi-squared test were used to compare continuous and categorical variables, respectively. Post hoc comparisons between groups were carried out using the Mann–Whitney *U* test and the chi-squared test. To test our hypothesis, we used the Pearson or the bivariate Spearman's rank correlation coefficient to analyze the relationship between HCV viral load and bilirubin levels among patients treated with ATV/rtv. Associations with p values of <0.05 were considered significant for comparisons between groups. Multivariate analysis consisted of a stepwise linear regression analysis.

Six hundred and forty-nine HIV/HCV-coinfected patients were included in the analysis. Forty patients (7.3%) were treated with ATV/rtv, 128 (23.4%) with LPV/rtv, 225 (41.2%) with EFV, 41 (7.5%) with NVP, and 112 (20.5%) received some other PI. Only nine patients who might have been included in the analysis and fulfilled the criteria for inclusion in the study were receiving darunavir/r. Given the low number of patients, we decided not to include them as an independent group for analysis The most significant characteristics are shown in Table 1.

Median HCV RNA levels of HCV genotypes 1/4 and 2/3 were 6.13 (5.6–6.7) log IU/ml and 5.7 (5.3–6.2) log IU/ml, respectively ($p<0.001$). HCV genotype 1/4 patients who received ATV/rtv had higher HCV RNA levels [6.57 (6.2–6.8) log IU/ml] than those receiving LPV/rtv [6.1 (5.5–6.5) log IU/ml], EFV [6.1 (5.6–6.4) log IU/ml], NVP [5.8 (5.5–5.9) log IU/ml], or another PI drug [6.1 (5.7–6.4) log IU/ml ($p=0.014$)]. However, this association was not found in patients bearing HCV genotype 2/3 [ATV/rtv: 5.74 (5.4–5.9) log IU/ml], LPV/rtv [5.7 (5.2–5.8) log IU/ml], EFV [5.8 (5.6–6) log IU/ml], NVP [5.3 (4.9–5.5) log IU/ml], and other PI drug [5.6 (5.4–5.9) log IU/ml, $p=0.34$].

Table 1. Baseline Population Characteristics

Characteristics	
N	649
Age (years), mean (SD)	40.8 (5.56)
Male gender, *n* (%)	537 (82.7)
AIDS criteria, *n* (%)	192 (29.6)
HbsAg positive, *n* (%)	8 (1.2)
Undetectable HIV viral load, *n* (%)	472 (72.7)
CD4 cell count (cells/mm^3), mean (SD)	518 (266)
HCV genotype 1/4, *n* (%)	452 (69.6)
Liver cirrhosis stage, *n* (%)	154 (23.7)
IL28B-CC genotype, *n* (%)[a]	115 (38.1)

[a]Available for 302 patients.

SD, standard deviation; AIDS, acquired immunodeficiency syndrome criteria in the past; HbsAg, hepatitis B surface antigen; IL28B, interleukin 28B.

The IL28B-CC genotype was associated with a higher HCV viral load than the non-CC genotype [6.3 (6.2–6.6) log IU/ml versus 6 (5.9–6.3) log IU/ml, $p=0.001$]. There were no differences in HCV viral load found on the basis of detectable HIV viral load [detectable, 6.1 (5.8–6.4) versus undetectable 6 (5.4–6.2), $p=0.247$] or liver fibrosis stage [advanced liver fibrosis: 6.2 (5.8–6.5) versus absence of liver fibrosis stage: 6.1 (5.9–6.3), $p=0.472$]. When HCV RNA viral loads were analyzed in terms of the drug used for ART, patients with IL28B-CC receiving ATV/rtv had higher viral loads than those receiving another drug [ATV/rtv: 7.3 (6.9–7.4) log IU/ml; LPV/rtv: 6.6 (6.2–6.8) log IU/ml; EFV: 6.6 (6.3–6.8) log IU/ml; other PI: 6.4 (6.2–6.9) log IU/ml, $p=0.038$]. Patients with IL28B non-CC receiving ATV had higher baseline HCV viral loads than those receiving another ART regimen [ATV/rtv: 6.15 (6–6.4) log IU/ml; LPV/rtv: 5.9 (5.8–6.2) log IU/ml; EFV: 5.8 (5.6–6) log IU/ml; other PIs: 5.9 (5.8–6.1) log IU/ml, $p=0.032$]. The mean ($\pm$SD) bilirubin level among those patients who received ATV/rtv was 2.31 ± 0.87. In these patients, HCV viral load correlated positively with bilirubin levels ($r=0.39$; $p=0.0129$).

Table 2 shows the linear regression model. Independent factors associated with HCV RNA levels were IL28B genotype

Table 2. Linear Regression Model for Hepatitis C Virus Baseline Viral Load in Patients Bearing the Hepatitis C Virus Genotype 1

Variable	*Condition*	*B*	p
IL28B	CC	0.7	0.033
ATV/rtv	Use	0.8	0.017
Liver fibrosis stage	Significant	0.075	0.884
HIV viral load	Undetectable	0.18	0.321

$R^2=0.22$.

B, adjusted coefficient; IL28B, interleukin 28B; ATV/rtv, atazanavir boosted with ritonavir; HIV, human immunodeficiency virus.

($B = 2.7$; $p = 0.033$) and use of ATV ($B = 2.8$; $p = 0.017$). R^2 was 0.22.

This is the first study to show the association between ATV/rtv use and HCV RNA viral load. Since a high HCV RNA viral load is a risk factor for nonresponse to treatment, this may be an important issue in conditioning the response to pegylated-interferon (PEG-IFN) with ribavirin (PEG-IFN/RBV) or to therapy based on NS3 protease inhibitors in HIV/HCV genotype 1-coinfected patients.[7–9] However, the relationship between ATV/rtv and treatment response has not yet been analyzed.

The reason ATV use might be associated with higher HCV RNA viral loads is unknown. *In vitro* studies have reported that BR and BV show antiviral activity against HIV, the herpes virus, and HCV.[3] More specifically, the antiviral action against HCV is due to both recombinant and endogenous NS3/4A protease from replicon microsomes being inhibited by BR and BV.[3] HO-1, on the other hand, potentiates interferon (IFN)-α, and so enhances antiviral activity.[2] Hyperbilirubinemia and ATV use are directly associated because of ATV's competing inhibitory activity against UGT1A1, responsible for BR conjugation in the liver. Inhibition may modify hemoglobin catabolism by suppressing various enzymes with antiviral activity against HCV (BR-R, BV-S, and HO-1) due to the enhancement of the resulting metabolite (BR) or reduced ISG activity (from the inhibition or downregulation of HO-1). There is no evidence that ATV blocks the activity of HO-1, in addition to inhibiting UGT1A1. However, a clinical trial is currently in progress whose secondary objective is to determine the effect of atazanavir-induced hyperbilirubinemia on HO-1 induction (NCT00916448), which may clarify this question. On the other hand, our study showed that NVP use was associated with lower HCV viral load, as has previously been reported by our group.[10]

Our study has several limitations. First, the number of patients with ATV-based therapy was relatively low. Second, the design was not a randomized study, which may have led to bias not being recognized. Third, no concomitant drugs were considered in the analysis. Fourth, although BR is closely related to ATV/rtv use, BR levels were not collected. Fifth, no concomitant diseases were analyzed in our study, and it is known that several liver pathologies may condition BR levels, as Gilbert's syndrome.

In conclusion, on the basis of our results, ATV-based therapy may be associated with higher HCV RNA viral loads among HIV/HCV-coinfected patients, a finding that could have an important impact on the outcome of HCV therapy. Studies are needed to confirm the relationship found in our study, as well to analyze this association in HCV treatment response.

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References

1. Choi J and Ou JHJ: Mechanism of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus. Am J Physiol Gastrointest Liver Physiol 2006;290:G847–851.
2. Lehmann E, EL-Tantawy WH, Ocker M, *et al.:* The heme oxygenase 1 product biliverdin interferes with hepatitis C virus replication by increasing antiviral interferon response. Hepatology 2010;51:398–404.
3. Zhu Z, Wilson AT, Luxon BA, *et al.:* Biliverdin inhibits hepatitis C virus NS3/4A protease activity: Mechanism for the antiviral effects of heme oxygenase? Hepatology 2010;52:1897–1905.
4. Fillebeen C, Rivas-Estilla AM, Bisaillon M, *et al.:* Iron inactivates the RNA polymerase NS5B and suppresses subgenomic replication of hepatitis C virus. J Biol Chem 2005;280:9049–9057.
5. Brierly C and Burchell B: Human UDP-glucoronosyl transferases: Chemical defence, jaundice and gene therapy. Bioessays 1993;15:749–754.
6. Neukam K, Mira JA, Ruiz-Morales J, *et al.:* Liver toxicity associated with antiretroviral therapy including efavirenz or ritonavir-boosted protease inhibitors in a cohort of HIV/hepatitis C virus co-infected patients. J Antimicrob Chemother 2011;66:2605–2614.
7. Dore GJ, Torriani FJ, Rodriguez-Torres M, *et al.:* Baseline factors prognostic of sustained virological response in patients with HIV-hepatitis C virus co-infection. AIDS 2007;21:1555–1559.
8. Poordad F, McCone J, Bacon BR, *et al.:* Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011;364:1195–1206.
9. Jaconson IM, Mchutchison JG, Dusheiko G, *et al.:* Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med 2011;364;25:2405–2416.
10. Mata RC, Mira JA, Rivero A, *et al.:* Nevirapine-based antiretroviral therapy is associated with lower plasma hepatitis C virus viral load among HIV/hepatitis C virus-coinfected patients. J AIDS Clinic Res 2010;1:110.

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