

Intracellular and Plasma Trough Concentration and Pharmacogenetics of Telaprevir

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ABSTRACT - PURPOSE: Triple therapy for HCV-1 infection consists in boceprevir or telaprevir, ribavirin and PEG-interferon. Telaprevir is a P-glycoprotein substrate and it is metabolized by CYP3A4/5. No data have been published on intracellular penetration of telaprevir. We determined peripheral blood mononuclear cells (PBMCs) and trough plasma S and R telaprevir isomers concentrations; moreover, we evaluated the influence of some single nucleotide polymorphisms (SNPs) on these pharmacokinetic data after 1 month of triple therapy in humans. **METHODS:** Plasma and intracellular telaprevir concentrations were determined at the end of dosing interval (C_{trough}) using ULPC-MS/MS validated methods; allelic discrimination was performed through real-time PCR. **RESULTS:** Median telaprevir C_{trough} plasma concentrations were 2579 ng/mL and 2233 ng/mL for the pharmacologically more active S, and R, enantiomers, respectively, with median S/R plasma ratio of 1.11. In PBMC, the medians were 6863 ng/mL and 1096 ng/mL for S and R, respectively, with median S/R being 5.73. The PBMC:plasma ratio for S was 2.59 for R. Plasma ribavirin concentrations were directly correlated with plasma S-telaprevir concentrations. In linear regression analysis, only *CYP24A1_rs2585428* SNP ($p=0.003$) and body mass index ($p=0.038$) were able to predict S-telaprevir PBMC concentrations. **CONCLUSIONS:** Our preliminary data could increase the understanding of mechanisms underlying telaprevir intracellular and plasma exposure, suggesting the implementation of pharmacogenetics in these drug kinetic studies.

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INTRODUCTION

For many years, "dual therapy" with pegylated (PEG) interferon α (IFN α) and ribavirin (RBV) was considered the standard treatment. The role of this drug consists in a host immunomodulation and in an indirect antiviral effect, since they do not target HCV genome or proteins(1). In 2011 the first direct-acting antivirals, boceprevir (BOC) and telaprevir (TLV) were approved by the United States Food and Drug Administration for use as "triple therapy" in combination with PEG-IFN α /RBV in patient affected by HCV-1(2). Both these direct-acting antivirals act on HCV NS3/4A protease and show two different isomeric forms (3, 4).

The main limitation of these treatments was the increase of some already known adverse events of double therapy, such as hemolytic anemia (5-7). This increased toxicity seems to be due to increased renal impairment risk by TLV, leading to reduced RBV elimination, thus elevated intracellular concentration, thereby, hemolysis(8, 9).

RBV, and other anti-HCV drugs, target on hepatocytes, but monitoring liver drug concentration is difficult due to biopsies feasibility; for this reason, the most easy-to-reach peripheral blood mononuclear cells (PBMCs) could be a valid "surrogate" to investigate intracellular drug concentrations (10-13).

Our group study showed that TLV plasma C_{trough} levels were influenced by ATP-binding cassette (ABC) B1 and B11 SNPs, with a potential influence on intracellular levels being the involvement of P-glycoprotein(14).

The aims of this retrospective study carried out after 1 month exposure to triple therapy of a cohort of HCV-1 genotype infected patients were to, first, determine TLV in plasma and, for the first time, in PBMCs, and second, evaluate the

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influence of single nucleotide polymorphisms (SNPs) in genes involved in RBV (15) and in TLV transport, also SNPs of vitamin D pathway, and their correlation with intracellular TLV.

METHODS

Characteristics of the study population

Chronic hepatitis C affected patients treated with PEG-IFN α 2a (180mg/week) plus RBV (15mg/Kg/day) for 4 weeks followed by TLV (1125mg/12h) enrolled at Amedeo di Savoia Hospital (Turin, Italy) between 2012 and 2014 were enrolled. Inclusion criteria were: HCV-1 genotype patients without major contraindication to IFN α , RBV and TLV therapy, 18 years old, no co-infection, no concomitant interacting drugs, no IFN, RBV and TLV modification up to week 4 of triple therapy and no concomitant vitamin D administration. Drop-out patients for any reason were excluded from this analysis.

Study protocol "HCV-GEN" was approved by the local Ethics Committee. A written informed consent for the study was obtained from each enrolled subject.

Pharmacogenetics analyses

DNA was extracted from blood using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Allelic discrimination analysis was performed using TaqMan assays (Applied Biosystems, Foster City, CA) through real-time PCR (BIORAD, Milano, Italia).

Plasma RBV and TLV concentrations measurement

RBV and TLV isomers plasma levels were determined from samples obtained at the end of dosing interval (C_{trough} , 12 h \pm 3 h) at week 4 of triple therapy. Patient samples (collected in the lithium-heparin tube, 7 mL) were centrifuged at 1500 rpm for 10 minutes at 4° C within 30 minutes from blood sampling and plasma was stored in criovials at -20° C before the analysis. RBV concentrations were determined using a high performance liquid chromatography system coupled with an ultraviolet determination (HLPC-UV), according to a chromatographic method previously published and routinely used in our laboratory (16).

TLV isomers concentrations were determined using a validated reverse phase ultra performance liquid chromatography tandem mass spectrometry method (ULPC-MS/MS)(4).

PBMCs TLV concentrations measurement

Intra-PBMC TLV isomers concentrations were measured through an UPLC-MS/MS coupled with On-Line Solid Phase Extraction validated method(17). Briefly, PBMC samples were isolated by density gradient using CPT vacutainers; the resulting extracts were centrifuged and 20 μ l of supernatants were directly injected into the OSM platform (Waters, Milford, MA, USA) for the SPE protocol.

The chromatographic separation was obtained on a BEH C18 1.7 μ m 2.1x150 mm column with a gradient of 2 mobile phases: A, water + NH $_4$ OH and B, acetonitrile:methanol 50:50 (v:v).

Statistical analyses

For descriptive statistics, continuous variables were summarized as median (IQR, interquartile range, 25th to 75th percentiles), categorical variables were described as frequency and percentage.

All of the SNPs were tested for Hardy-Weinberg equilibrium by the χ^2 test, in order to determine the observed genotype frequencies. Linkage Disequilibrium (LD) was evaluated with Haploview 4.2 software (Cambridge, Massachusetts, USA). We measured LD between two SNPs using the statistic D' : $|D'|$ of 1 indicates complete LD, 0 corresponds to absence of LD.

Correlation between continuous variables was performed by Pearson test (r coefficient), considering the level of statistical significance (p -value) $<$ 0,05. Kruskal-Wallis and Mann-Whitney tests have been used to compare plasma concentration between different genotypes (p $<$ 0,05). Any predictive power of considerable variables was finally evaluated through univariate (p $<$ 0,2) and multivariate (p $<$ 0,05) linear regression analyses (OR, odd ratio; IC: interval of confidence at 95%).

All the statistic tests were performed with IBM SPSS Statistics 20.0 per Windows (Chicago, Illinois, USA).

RESULTS

Characteristics of patients

Ten patients were included in this analysis: their characteristics were collected in Table 1.

Pharmacogenetics Analyses

All the studied SNPs were in Hardy-Weinberg equilibrium; variant allele frequencies were resumed in Table 2.

Table 1. Demographic and clinical characteristics of HCV-1 patients

Number of patients, n	10
Male, n (%)	9 (90)
Naive patients, n (%)	1 (10)
Previous treatment outcome, n (%)	
-null responders	2 (20)
-partial responders	0 (0)
-relapsers	7 (70)
Median age (IQR)	43 (37-55)
Median BMI, Kg/m ² (IQR)	27.6 (24.89-29.84)
Caucasians, n (%)	10 (100)
Median HCV-RNA at baseline, log IU/mL (IQR)	577653.5 (180578-4122331)
Median ALT at baseline, IU/L (IQR)	79.5 (44-151)
Metavir score, n (%)	
F0	2 (20)
F1	3 (30)
F2	0 (0)
F3	2 (20)
F4	3 (30)
RBV dose, n (%)	
1200 mg/day	2 (20)
1000 mg/day	5 (50)
800 mg/day	1 (1)

IQR, interquartile range; BMI, body mass index; ALT, alanine aminotransferase; Values in brackets are range of standard deviation. No differences concerning demographic, racial, physical characteristics and biochemical parameters (Table 1) were observed among genetically defined groups.

Only *IL28B_rs12979860* and *rs8099917* SNPs resulted in complete LD ($D^2=1$) in our population. None of the analyzed SNPs were statistically associated with TLV PBMC exposure.

C_{trough} Analyses

Median TLV plasma C_{trough} was 2579 ng/mL (IQR 1476-2903 ng/mL) of S and 2233 ng/mL (IQR 1288-2488 ng/mL) of R isomers. Median S/R plasma ratio was 1.11 (IQR 1.01-1.27).

Median value obtained for intra-PBMCS levels was 6863 ng/mL (IQR 5383-7908 ng/mL) and 1096 ng/mL (IQR 747-1976 ng/mL) for R. Median intra-PBMCS/R ratio was 5.73 (IQR 3.68-8.10), whereas S-TLV in PBMC/plasma ratio was 2.59 (IQR 2.29-4.30). A significant correlation between RBV plasma concentration and plasma S-TLV ($p=0.708$; $p=0.022$), R ($p=0.764$; $p=0.010$) and S-TLV in PBMC/plasma ratio ($p=-0.702$;

$p=0.024$) was found; plasma R-TLV correlated with S-TLV in PBMC/plasma ratio ($p=-0.721$; $p=0.019$). Plasma S/R was significantly correlated with S-TLV in PBMC/plasma ratio ($p=-0.759$; $p=0.011$).

In linear regression analysis, only *CYP24A1_rs2585428* SNP ($p=0.003$; OR:-2567; IC95%:-3933--1202) and body mass index (BMI, $p=0.038$; OR:263; IC95%:19-507) were predictable of the intra-PBMC S levels.

DISCUSSION

TLV specifically blocks HCV polyprotein processing by covalently and reversibly binding to the active-site serine of viral NS3-4A protease (18). It is metabolized in the liver through hydrolysis, oxidation and reduction reactions by CYP3A4. TLV predominant inactive metabolites

Table 2. Variants allele frequencies in patients described in Table 1.

SNPs	Wild-type allele: %	Mutant allele: %
<i>ABCB1</i> +3435 C>T	56.85 (C)	43.15 (T)
<i>ABCB1</i> +1236 C>T	56.8 (C)	43.2 (T)
<i>ABCB1</i> 2677 G>T	54.55 (G)	45.45 (T)
<i>ABCB11</i> 1131 T>C	27.25 (T)	72.75 (C)
<i>IL28B</i> rs12979860 T>C	54.55 (T)	45.45 (C)
<i>IL28B</i> rs12980275 G>A	59.1 (G)	40.9 (A)
<i>IL28B</i> rs8099917 T>G	59.05 (T)	40.95 (G)
<i>ITPA</i> rs1127354 C<A	84.05 (C)	15.95 (A)
<i>ITPA</i> rs7072101A>C	88.65 (A)	11.35 (C)
<i>ITPA</i> rs6051702 A>C	86.35 (A)	13.65 (C)
<i>NT5C2</i> rs10883841 T>C	90.9 (T)	9.1 (C)
<i>HNF4a</i> rs1884613 C>G	81.85 (C)	18.15 (G)
<i>SLC28A2</i> -146 A>T	34.05 (A)	56.95 (T)
<i>SLC28A2</i> 225 A>C	36.35 (A)	63.65 (C)
<i>SLC28A2</i> 124 C>T	47.7 (C)	52.3 (T)
<i>SLC28A3</i> 338 T>C	97.75 (T)	2.25 (C)
<i>SLC29A1</i> rs747199 C>G	81.85 (C)	18.15 (G)
<i>SLC29A1</i> rs760370 A>G	56.8 (A)	43.2 (G)
<i>CYP27B1</i> 2838 C>T	74.95 (C)	25.05 (T)
<i>CYP27B1</i> 1260 G>T	25.05 (G)	74.95 (T)
<i>CYP24A1</i> rs2248359 T>C	40.85 (T)	59.15 (C)
<i>CYP24A1</i> rs2585428 A>G	43.2 (A)	56.8 (G)
<i>CYP24A1</i> rs927650 C>T	45.45 (C)	54.55 (T)
<i>VDR</i> <i>ApaI</i> C>A	70.45 (C)	29.55 (A)
<i>VDR</i> <i>TaqI</i> T>C	56.85 (T)	43.15 (C)
<i>VDR</i> <i>BsmI</i> G>A	75 (G)	25 (A)
<i>VDR</i> <i>FokI</i> T>C	27.3 (T)	72.7 (C)
<i>VDR</i> <i>Cdx2</i> A>G	20.4 (A)	79.6 (G)

ABCB1/11, ATP-binding cassette 1/11; IL28B, interleukin 28B; ITPA, inosine triphosphate pyrophosphatase; NT5C2, cytosolic 5'-nucleotidase 2; HNF4 α , hepatocyte nuclear factor 4; SLC 28A2/3 and 29A1, solute c carrier 28A2/3 and 29A1; CYP 27B1/24A1, cytochrome P450 27B1/24A1; VDR, vitamin D receptor.

are R-diastereomer (30-fold less active), pyrazinoic acid and a reduced α -ketoamide bond one(19). Weiss *et al.* showed that this drug is a moderate inhibitor and substrate of P-glycoprotein and an strong inducer of ABCG2 (20). TLV is eliminated in faeces (82%), expired air (9%) and urine (1%)(19). PEG-IFN α increases TLV steady state (22%), C_{max} and AUC (30-40%)(21).

In this paper, we show, for the first time, that despite a lack stereoselectivity in plasma, intra-PBMC levels of S were about 5-fold greater than the R enantiomer. Furthermore, we confirmed the observation (22) that RBV plasma levels were

directly correlated with S-TLV plasma concentrations, consequently with S-TLV in PBMC/plasma ratio following the administered fix-dose regimen. Conversely, we found that RBV is also correlated with R-isomer plasma exposure. This aspect has not yet been fully clarified, although several hypotheses have been formulated (6, 23): probably, the glomerular filtration activity reduction, due to TLV administration, could lead to minor RBV excretion, thus its higher plasma concentrations.

In linear regression analysis, we found that BMI and a polymorphism in *CYP24A1* gene were

able to predict S intracellular levels. Particularly, BMI was a positive predictor factor, whereas *CYP24A1* SNP was a negative one.

Many immune mechanisms have been identified for spontaneous viral clearance and for the success of antiviral therapy(24, 25).

The activated vitamin D is an important immunomodulator and most of its biological effects are mediated through vitamin D receptor (VDR)(26).

Polymorphisms in genes of enzymes involved in vitamin D inactivation are important for prostate cancer prognosis(27) and HCV-2/3PEG-IFN α /RBV treatment outcomes; particularly, *CYP24A1_rs2585428GG* positively predicts therapeutic failure and negatively early virological response(28).

Intra-PBMCs pharmacokinetics of TLV deserve further investigation to better understand the role of intracellular drug concentrations in determining treatment efficacy and toxicity.

TLV therapeutic drug monitoring could be useful tool for the management of HCV infected patients(29) and the knowledge of intracellular penetration mechanism may be the key factor to improve treatment outcome.

Also concerning BOC, a study by our group suggested a positive and significant correlation between plasma and PBMC levels. Moreover, we revealed that plasma levels of S-BOC active isomer and a polymorphism in the vitamin D receptor were able to predict S-intracellular exposure, whereas SNPs in aldo-keto reductase 1, breast cancer resistance protein1 and solute carrier family 28 genes predicted S-TLV in PBMC/plasma ratio(30).

Our preliminary data could increase the understanding of mechanisms underlying TLV intracellular and plasma exposure, suggesting the implementation of pharmacogenetics in these drug kinetic studies.

Sample size is the main limitation of this study, thus these tests have to be confirmed in a wider cohort of patients. It could be useful to evaluate intracellular RBV concentration data to better understand the mechanisms underlying its interaction with TLV.

To date, in several countries, new Direct-acting antivirals have been approved, opening the new era of IFN-free, reducing duration and increasing success rate therapy.

A similar approach should be useful for the study of these new drugs, to promptly understand pharmacogenetic factors involved in the regulation of their plasma and intracellular pharmacokinetics.

Conflict of interest: The authors declare no competing financial interest.

REFERENCES

1. Perry CM. Telaprevir: a review of its use in the management of genotype 1 chronic hepatitis C. *Drugs*. 2012 Mar 26;72(5):619-41.
2. FDA. Center for Drug Evaluation and Research: 202258Orig1s000 – BOCEPREVIR, 2011. 2011; 2011:[Available from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/202258Orig1s000MedR.pdf.
3. Kim DY, Ahn SH, Han KH. Emerging Therapies for Hepatitis C. *Gut Liver*. 2014 Sep;8(5):471-9.
4. D'Avolio A, De Nicolo A, Agnesod D, Simiele M, Mohamed Abdi A, Dilly Penchala S, et al. A UPLC-MS/MS method for the simultaneous plasma quantification of all isomeric forms of the new anti-HCV protease inhibitors boceprevir and telaprevir. *J Pharm Biomed Anal*. 2013 May 5;78-79:217-23.
5. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med*. 2011 Jun 23;364(25):2405-16.
6. De Nicolo A, Boglione L, Ciancio A, Cusato J, Strona S, Cardellino CS, et al. Telaprevir-S isomer enhances ribavirin exposure and the ribavirin-related haemolytic anaemia in a concentration-dependent manner. *Antiviral Res*. 2014 Jun 20.
7. Boglione L, De Nicolo A, Cusato J, Cariti G, Di Perri G, D'Avolio A. Significant early higher ribavirin plasma concentrations in patients receiving a triple therapy with pegylated interferon, ribavirin and telaprevir. *J Viral Hepat*. 2014 Apr;21(4):260-3.
8. Mauss S, Hueppe D, Alshuth U. Renal impairment is frequent in chronic hepatitis C patients under triple therapy with telaprevir or boceprevir. *Hepatology*. 2013 Jan;59(1):46-8.
9. Hammond K, Jimmerson L, MacBrayne C, Ray M, Bushman L, Burton J, et al. PP_02 Increased Plasma and Intracellular Ribavirin Concentrations Associated with Telaprevir Use. *Reviews in antiviral therapy & infectious diseases*. 2013;3:26.
10. D'Avolio A, Carcieri C, Cusato J, Simiele M, Calcagno A, Allegra S, et al. Intracellular accumulation of atazanavir/ritonavir according to plasma concentrations and OATP1B1, ABCB1 and PXR genetic polymorphisms. *J Antimicrob Chemother*. 2014 Jul 4.
11. D'Avolio A, Pensi D, Baietto L, Di Perri G. Therapeutic drug monitoring of intracellular anti-infective agents. *J Pharm Biomed Anal*. 2014 Apr 3.
12. De Francia S, D'Avolio A, Ariaudo A, Pirro E, Piccione F, Simiele M, et al. Plasma and intracellular imatinib concentrations in patients

- with chronic myeloid leukemia. *Ther Drug Monit*. 2013 Jun;36(3):410-2.
13. De Nicolo A, Simiele M, Calcagno A, Abdi AM, Bonora S, Di Perri G, et al. Intracellular antiviral activity of low-dose ritonavir in boosted protease inhibitor regimens. *Antimicrob Agents Chemother*. 2014 Jul;58(7):4042-7.
 14. Jessica Cusato, Sarah Allegra, Amedeo De Nicolò, Lucio Boglione, Giovanna Fatiguso, Giuseppe Cariti, et al. *ABCB11* and *ABCB1* gene polymorphisms impact on telaprevir pharmacokinetic at one month of therapy. *Biomedicine & Pharmacotherapy* 2014;accepted.
 15. Allegra S, Cusato J, Nicolò AD, Boglione L, Gatto A, Cariti G, et al. Role of pharmacogenetic in ribavirin outcome prediction and pharmacokinetics in an Italian cohort of HCV-1 and 4 patients. *Biomedicine & Pharmacotherapy* 2014 6/11/2014;11.
 16. D'Avolio A, Ibanez A, Sciandra M, Siccardi M, de Requena DG, Bonora S, et al. Validation of liquid/liquid extraction method coupled with HPLC-UV for measurement of ribavirin plasma levels in HCV-positive patients. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2006 May 1;835(1-2):127-30.
 17. De Nicolò A, Abdi AM, Boglione L, Baietto L, Pensi D, Allegra S, et al. UPLC-MS/MS coupled with automated on-line SPE method for the isomer-specific quantification of the first-generation anti-HCV protease inhibitors in peripheral blood mononuclear cells. *Journal of Chromatography A*. 2014;submitted.
 18. Perni RB, Almquist SJ, Byrn RA, Chandorkar G, Chaturvedi PR, Courtney LF, et al. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother*. 2006 Mar;50(3):899-909.
 19. Vertex. INCIVEK (telaprevir). United States prescribing information 2012. Cambridge, MA, USA 2012 [updated June 2012; July 2012]; Available from: http://pi.vrtx.com/files/uspi_telaprevir.pdf.
 20. Weiss J, Becker JP, Haefeli WE. Telaprevir is a substrate and moderate inhibitor of P-glycoprotein, a strong inducer of ABCG2, but not an activator of PXR in vitro. *Int J Antimicrob Agents*. 2014 Feb;43(2):184-8.
 21. Marcellin P, Forns X, Goeser T, Ferenci P, Nevens F, Carosi G, et al. Telaprevir is effective given every 8 or 12 hours with ribavirin and peginterferon alfa-2a or -2b to patients with chronic hepatitis C. *Gastroenterology*. 2011 Feb;140(2):459-68 e1; quiz e14.
 22. De Nicolo A, Boglione L, Ciancio A, Cusato J, Strona S, Cardellino CS, et al. Telaprevir-S isomer enhances ribavirin exposure and the ribavirin-related haemolytic anaemia in a concentration-dependent manner. *Antiviral Res*. 2014 Sep;109:7-14.
 23. Tempestilli M, Lionetti R, D'Offizi G, Montalbano M, Giaffreda A, Fazio S, et al. Increased plasma concentration of ribavirin as a result of renal dysfunction in hepatitis C virus patients treated with telaprevir. *Hepatology*. 2014 Sep;60(3):1109-10.
 24. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. 2009 Sep 17;461(7262):399-401.
 25. Thimme R, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, et al. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci U S A*. 2002 Nov 26;99(24):15661-8.
 26. Poon AH, Gong L, Brasch-Andersen C, Litonjua AA, Raby BA, Hamid Q, et al. Very important pharmacogene summary for VDR. *Pharmacogenet Genomics*. 2012 Oct;22(10):758-63.
 27. Holt SK, Kwon EM, Koopmeiners JS, Lin DW, Feng Z, Ostrander EA, et al. Vitamin D pathway gene variants and prostate cancer prognosis. *Prostate*. 2010 Sep 15;70(13):1448-60.
 28. Cusato J, Allegra S, Boglione L, De Nicolo A, Baietto L, Cariti G, et al. Vitamin D pathway gene variants and HCV-2/3 therapy outcomes. *Antivir Ther*. 2014 Oct 3.
 29. Furusyo N, Ogawa E, Murata M, Toyoda K, Ohnishi H, Eiraku K, et al. Therapeutic drug monitoring of telaprevir in chronic hepatitis C patients receiving telaprevir-based triple therapy is useful for predicting virological response. *J Antimicrob Chemother*. 2014 Feb;69(2):483-90.
 30. Cusato J, Allegra S, Nicolò AD, Boglione L, Fatiguso G, Abdi AM, et al. Intracellular accumulation of boceprevir according to plasma concentrations and pharmacogenetics. *International Journal of Antimicrobial Agents*. 2015