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Short Communication

Intracellular accumulation of boceprevir according to plasma concentrations and pharmacogenetics

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ABSTRACT

Boceprevir (BOC) is a directly-acting antiviral agent for the treatment of hepatitis C virus genotype 1 (HCV-1) infection. It is a mixture of two stereoisomers, the inactive R and the active S isomers. No data have previously been published on BOC intracellular accumulation. In this study, BOC isomer concentrations in peripheral blood mononuclear cells (PBMCs) and plasma were determined. The influence of various single nucleotide polymorphisms (SNPs) on plasma and intracellular drug exposure at Week 4 of triple therapy were also evaluated. Plasma and intracellular BOC concentrations were determined at the end of the dosing interval (C_{trough}) using a UPLC-MS/MS validated method. Allelic discrimination was performed through real-time PCR. Median plasma concentrations were 65.97 ng/mL for the S isomer and 36.31 ng/mL for the R isomer; the median S/R plasma concentration ratio was 1.66. The median PBMC concentration was 2285.88 ng/mL for the S isomer; the R isomer was undetectable within PBMCs. The median S isomer PBMC/plasma concentration ratio was 28.59. A significant positive correlation was found between plasma and PBMC S isomer concentrations. *ABCB1* 1236, *SLC28A2* 124 and *IL28B* rs12979860 SNPs were associated with the S isomer PBMC/plasma concentration ratio. In regression models, S isomer plasma levels and *FokI* polymorphism were able to predict S isomer intracellular exposure, whereas SNPs in *AKR1*, *BCRP1* and *SLC28A2* predicted the S isomer PBMC/plasma concentration ratio. No similar data regarding BOC pharmacogenetics and pharmacokinetics have been published previously. This study adds a novel and useful overview of the pharmacological properties of this drug.

1. Introduction

Approval of the first-generation protease inhibitors boceprevir (BOC) and telaprevir (TVR) in 2011 changed the standard of care for hepatitis C virus genotype 1 (HCV-1) infection from dual to triple therapy. BOC or TVR plus ribavirin (RBV) and pegylated interferon-alfa (PEG-IFNα) is associated with an improvement in the sustained virological response (SVR) rate both for naïve and experienced HCV-1 patients [1]. BOC-based triple therapy consists of a 48-week treatment duration and it is a good choice for those unable to tolerate the adverse effects of TVR [2].

BOC is a peptidomimetic α-ketoamide that is able to reversibly bind the serine-139 active site of HCV NS3/4A protease, halting viral replication. This drug is a mixture of two interconvertible diastereoisomers: SCH-534128 (the S isomer) is 41–130-fold more active and has ca. 2-fold higher systemic exposure than SCH-534129 (the R isomer) [3]. BOC is metabolised by cytochromes P450 3A4/5 and aldo-keto reductase (AKR) 1C2/3; the keto-reduced metabolites do not have antiviral activity and they are the most abundant metabolite present in the bloodstream [4]. BOC is mainly eliminated by the liver (80%) and a small percentage by the kidney (10%) [3]. No data have been published on the intracellular accumulation of BOC.

In this study, plasma and intracellular BOC concentrations were determined using the easily accessible peripheral blood mononuclear cells (PBMCs) as a model for drug penetration. Moreover, the influence of various single nucleotide polymorphisms (SNPs) in genes involved in BOC and RBV transport and the vitamin D

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pathway on BOC exposure at Week 4 of triple therapy were as evaluated in a cohort of HCV-1-infected patients.

2. Materials and methods

2.1. Characteristics of the study population

Chronic HCV-1-affected patients treated with leukocyte IFN or PEG-IFNα 2a or 2b plus RBV (15 mg/kg/day) for 4 weeks followed by the addition of BOC (800 mg every 8 h) referred to Amedeo di Savoia Hospital (Turin, Italy) between 2012 and 2014 were enrolled in this study. Inclusion criteria were as follows: HCV-1-infected patients without major contraindication to IFN/RBV/BOC therapy; age ≥18 years; no co-infection; no concomitant interacting drugs; no BOC/RBV/IFN modification up to Week 4 of triple therapy; and no concomitant vitamin D administration. Drop-out patients for any reason were excluded from the analysis.

The study protocol 'HCV-GEN' was approved by the local Ethics Committee (San Luigi Hospital, University of Turin, Turin, Italy). Written informed consent for the study was obtained from each enrolled subject.

2.2. Pharmacogenetic analyses

DNA was extracted from blood using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA). Allelic discrimination was performed using *Taq*Man® assays (Applied Biosystems, Foster City, CA) by real-time PCR (Bio-Rad, Milan, Italy).

2.3. Measurement of plasma and peripheral blood mononuclear cell boceprevir concentrations

BOC concentrations at the end of the dosing interval (C_{trough}) (8 ± 1 h) were analysed at Week 4 of triple therapy. Drug concentrations were determined using a reversed-phase ultra performance liquid chromatography tandem mass spectrometry method (UPLC-MS/MS) according to a fully validated method [5]. PBMC-associated S and R BOC isomer concentrations were measured through a UPLC MS/MS coupled with online solid phase extraction validated method (unpublished data). Automatic determination of cell number and median cellular volume was used to normalise analytical data, such as drug amounts in cell pellets, as described by Simiele et al. [6].

2.4. Statistical analyses

For descriptive statistics, continuous variables were summarised as the median [interquartile range (IQR)] and 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 (Broad Institute of MIT and Harvard, Cambridge, MA). LD between two SNPs was measured using the statistic D'. A $|D'|$ of 1 indicates complete LD, whereas 0 corresponds to no LD.

Correlation between continuous variables was analysed by Pearson's test (r coefficient), with a P-value of <0.05 considered statistically significance. Kruskal–Wallis and Mann–Whitney tests were used to compare plasma concentrations between different genotypes ($P < 0.05$). Any predictive power of the considered variables was finally evaluated through univariate ($P < 0.2$) and multivariate ($P < 0.05$) linear regression analyses [odds ratio (OR) and 95% confidence interval (CI)].

All of the statistical tests were performed using IBM SPSS Statistics for Windows v.20.0 for Windows (IBM Corp., Armonk, NY).

Table 1
Demographic and clinical characteristics of hepatitis C virus genotype 1 (HCV-1) patients.

Characteristic	
No. of patients	22
Male [*n* (%)]	17 (77.3)
Naïve patients [*n* (%)]	8 (36.4)
Previous treatment outcome [*n* (%)]	
Null responders	11 (50.0)
Partial responders	1 (4.5)
Relapsers	2 (9.1)
Age (years) [median (IQR)]	45.5 (43.25–54.75)
BMI (kg/m^2) [median (IQR)]	25.98 (24.4–28.8)
Caucasians [*n* (%)]	21 (95.5)
HCV-RNA at baseline (log IU/mL) [median (IQR)]	1 382 110 (895 304.5–5 478 407)
ALT at baseline (IU/L) [median (IQR)]	115.5 (72.0–160.75)
Metavir score [*n* (%)]	
F0	0 (0.0)
F1	3 (13.6)
F2	4 (18.2)
F3	5 (22.7)
F4	10 (45.5)
IFN dose [*n* (%)]	
6 MUI 3 times a week	1 (4.5)
80 μg/week	2 (9.1)
100 μg/week	6 (27.3)
120 μg/week	7 (31.8)
150 μg/week	4 (18.2)
180 μg/week	2 (9.1)
IFN type [*n* (%)]	
PEG-IFNα 2a	2 (9.1)
PEG-IFNα 2b	19 (86.4)
Leukocyte	1 (4.5)
RBV dose [*n* (%)]	
1400 mg/day	1 (4.5)
1200 mg/day	11 (50.0)
1000 mg/day	8 (36.4)
800 mg/day	2 (9.1)

IQR, interquartile range; BMI, body mass index; ALT, alanine aminotransferase; IFN, interferon; PEG, pegylated; MUI, million international units; RBV, ribavirin.

3. Results

3.1. Patient characteristics

Twenty-two patients were included in the analysis. Their characteristics are summarised in Table 1. No differences with regard to demographic, racial and physical characteristics or biochemical parameters (haemoglobin, platelet count, alanine amino transferase, serum HCV-RNA level) were observed among genetically defined groups.

3.2. Pharmacokinetic analyses

The median BOC C_{trough} plasma concentration was 65.97 ng/mL (IQR 44.68–117.94 ng/mL) for the S isomer and 36.31 ng/mL (IQR 25.24–85.32 ng/mL) for the R isomer. The median S/R plasma concentration ratio was 1.66 (IQR 1.53–2.05).

The median value obtained for S isomer PBMC levels was 2285.88 ng/mL (IQR 1654.43–3608.55 ng/mL) (Fig. 1A); the R isomer was undetectable in PBMC samples (unpublished data). The median S isomer PBMC/plasma concentration ratio was 28.59 (IQR 21.64–43.55) (Fig. 1B). A significant positive correlation was found between plasma and intracellular S isomer concentrations ($r = 0.673$; $P = 0.001$) (Fig. 1C) and between plasma R isomer and intracellular S isomer concentrations ($r = 0.687$; $P < 0.001$) (Fig. 1D).