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Short Communication

Entecavir plasma concentrations are inversely related to HBV-DNA decrease in a cohort of treatment-naïve patients with chronic hepatitis B

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ABSTRACT

The role of therapeutic drug monitoring (TDM) of entecavir (ETV) in the treatment of patients affected by chronic hepatitis B (CHB) has not yet been defined. Here we present an interim analysis regarding the role of ETV TDM in a prospective cohort of treatment-naïve patients with CHB who received this treatment. The results from 40 patients consecutively enrolled at our centre from 2010 to 2013 are described. The primary endpoint was the evaluation of the role of ETV plasma concentrations in the kinetics of hepatitis B virus (HBV) DNA decrease. Minimum ETV concentrations (C_{trough}) were measured every month after the start of therapy for the first 3 months and then every 6 months. The main result of the pharmacokinetic analysis was the significant inverse correlation of ETV concentration after 1 month of treatment and HBV-DNA decrease after 3 months of treatment ($r = -0.624$; $P < 0.001$). This correlation was also confirmed when stratifying patients on the basis of viral genotypes: A ($r = -0.719$; $P = 0.003$); C ($r = -0.917$; $P = 0.007$); and D ($r = -0.760$; $P = 0.007$). Possible explanations for this phenomenon could involve interpatient differences in liver conditions (tissue damage or inflammation) and/or genetic variability in specific drug transporters. Further investigations are needed to confirm these results quantifying ETV concentration in peripheral blood mononuclear cells as well as in a larger cohort.

1. Introduction

Chronic hepatitis B (CHB) affects ca. 350 million people worldwide and is a major cause of cirrhosis, liver failure and hepatocellular carcinoma (HCC) [1].

Treatment for hepatitis B e-antigen (HBeAg)-negative CHB patients includes two different approaches: long-term therapy with oral nucleos(t)ide analogues (NAs), based on serum hepatitis B virus (HBV) DNA suppression; and finite-duration therapy with standard or pegylated interferon alfa (peg-IFNα) [2]. However, IFN treatment leads to poor virological response (30–40%) with a high relapse rate and is limited by multiple side effects, whereas the major problem with NA treatment is the need for long-term (indefinite length) therapy and the risk of drug resistance [3].

Currently, the most used NAs are entecavir (ETV) and tenofovir disoproxil fumarate (TDF); however, hepatitis B surface antigen (HBsAg) loss is very rare in HBeAg-negative patients [2]. HBsAg seroconversion was reported in 3–7% of patients treated with peg-IFN, in 0.5–3% of HBeAg-positive patients treated with NAs, and in 4% and 0% of HBeAg-negative patients treated with peg-IFN and NAs, respectively [2]. HBV-DNA suppression is a virological endpoint achieved in the majority of patients with ETV or TDF administration [4]. Monitoring of quantitative HBsAg (qHBsAg) serum kinetics during treatment with NAs evidenced a small decrease in comparison with IFN therapy, even when HBV-DNA is undetectable, and the achievement of HBsAg loss could only be gained after a very long time of drug intake [5,6]. Knowledge of qHBsAg kinetics during NA use could be interesting in order to understand the time to achieve a long-term outcome of HBsAg loss.

Nevertheless, the role of therapeutic drug monitoring (TDM) of NAs in HBV treatment is still undefined. The aim of the present prospective study was to evaluate the role of ETV plasma concentrations in the kinetics of HBV-DNA decrease in a cohort of treatment-naïve patients affected by active HBeAg-negative CHB.

2. Methods

2.1. Patients

Treatment-naïve HBeAg-negative patients with active CHB treated with ETV from 2010 to 2013 at the University Hospital Amedeo di

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Savoia (Turin, Italy) were prospectively included in this pharmacokinetic study. Inclusion criteria were: active CHB, defined as HBV-DNA >10,000 IU/mL and alanine aminotransferase (ALT) level >50 U/L; HBeAg-negative status; and naïve for previous antiviral therapies. Exclusion criteria were: co-infection with human immunodeficiency virus (HIV), hepatitis C virus (HCV) or hepatitis D virus (HDV); HBeAg-positive status; decompensated cirrhosis; and presence of HCC. The following tests were performed before the start of therapy: evaluation of liver stiffness by FibroScan®; liver ultrasound; ALT level, qHBsAg and HBV-DNA quantification; and HBV genotype testing.

The included patients underwent ETV therapy at the standard dose of 0.5 mg once daily. The primary endpoint was the evaluation of the role of ETV plasma concentrations in the kinetics of HBV-DNA decrease. Secondary endpoints included evaluation of serological and biochemical response and the role of HBV genotypes according to ETV plasma concentration.

After the beginning of treatment, HBV-DNA, qHBsAg, ALT levels and minimum ETV concentration (C_{trough}) were measured monthly for the first 3 months and then every 6 months. This study was performed in accordance with the indications of the Ethics Committee of Hospital Amedeo di Savoia, after written informed consent was obtained.

2.2. Assays

Serum HBV-DNA levels were quantified by real-time PCR using a COBAS® AmpliPrep/COBAS® *Taq*Man® HBV Test v.2.0 (Roche Molecular Systems Inc., Branchburg, NJ). HBV genotypes were determined using an INNO-LiPA reverse hybridisation assay (Innogenetics N.V, Ghent, Belgium). HBsAg, HBeAg and hepatitis B e antibody (anti-HBe) were detected using an Elecsys instrumental platform (Roche Diagnostics, Milan, Italy). qHBsAg test was performed using ARCHITECT HBsAg qualitative assay (Abbott Diagnostics, Dublin, Ireland) with a dynamic range of 0.05–250.0 IU/mL; qHBsAg values >250.0 IU/mL were subsequently serially diluted at 1:100 and were re-tested until falling within the dynamic range.

2.3. Blood sampling and entecavir quantification

Blood samples were collected 24 h (±2 h) after drug intake, immediately before the next administration (C_{trough}). Samples were collected in lithium heparin tubes (7 mL) and were centrifuged at 1400 × *g* for 10 min at 4 °C. Plasma was stored at –20 °C until analysis (no longer than 1 month). Quantification of ETV was performed following a previously validated ultra high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) method [7].

2.4. Statistical analysis

Data were assessed for normality by Shapiro–Wilk test. Binomial groups were compared using a Mann–Whitney test. To evaluate a possible correlation between continuous data, a Pearson correlation test was adopted. Finally, a receiver operating characteristic (ROC) curve analysis was conducted to determine concentration cut-off values. Independent predictivity of factors associated with HBV-DNA decay kinetics was evaluated through a multivariate linear regression.

All statistical analyses were performed using IBM SPSS Statistics for Windows v.22.0 (IBM Corp., Armonk, NY).

3. Results

3.1. Baseline description

Forty CHB patients were included in the analysis. Baseline characteristics of the patients are reported in Table 1. There were 32 male patients (80.0%) and the median patient age was 39.5 years [interquartile range (IQR) 31.5–51.0 years].

Table 1
Baseline characteristics of study population ($n = 40$).

Characteristic	
Age (years) [median (IQR)]	39.5 (31.5–51.0)
Male sex [*n* (%)]	32 (80.0)
Route of transmission [*n* (%)]	
Intravenous drug use	5 (12.5)
Transfusion	4 (10.0)
Sexual	11 (27.5)
Family history of HBV	7 (17.5)
Unknown	13 (32.5)
Geographical origin [*n* (%)]	
Italy	19 (47.5)
Eastern Europe	7 (17.5)
Africa	7 (17.5)
China	6 (15.0)
South America	1 (2.5)
HBV genotype [*n* (%)]	
A	15 (37.5)
B	3 (7.5)
C	5 (12.5)
D	11 (27.5)
E	5 (12.5)
F	1 (2.5)
Liver stiffness (kPa) [median (IQR)]	6.8 (6.5–10.1)
qHBsAg (log IU/mL) [median (IQR)]	4.17 (3.95–4.43)
HBV-DNA (log IU/mL) [median (IQR)]	7.19 (5.73–8.23)
ALT (U/L) [median (IQR)]	89 (76.2–122)

IQR, interquartile range; HBV, hepatitis B virus; qHBsAg, quantitative hepatitis B surface antigen; ALT, alanine aminotransferase.

The frequencies of viral genotypes A, B, C, D, E and F were 15 (37.5%), 3 (7.5%), 5 (12.5%), 11 (27.5%), 5 (12.5%) and 1 (2.5%), respectively. Median liver stiffness was 6.8 kPa (IQR 6.5–10.1 kPa); seven patients (17.5%) had compensated cirrhosis. The Metavir score was F0 in 2 cases (5.0%), F1 in 26 cases (65.0%), F2 in 7 cases (17.5%), F3 in 3 cases (7.5%) and F4 in 2 cases (5.0%). The median baseline ALT level was 89 U/L (IQR 76.2–122 U/L).

The median baseline log HBV-DNA and qHBsAg were 7.19 log IU/mL (IQR 5.73–8.23 log IU/mL) and 4.17 log IU/mL (IQR 3.95–4.43 log IU/mL), respectively. After 1, 2 and 3 months of treatment, log HBV-DNA and qHBsAg, respectively, were as follows (in log IU/mL): 5.65 (IQR 4.76–6.28) and 4.01 (IQR 3.88–4.26) at 1 month; 4.37 (IQR 3.80–5.60) and 3.94 (IQR 3.81–4.08) at 2 months; and 3.96 (IQR 0.00–4.89) and 3.94 (IQR 3.65–4.02) at 3 months.

3.2. Entecavir plasma concentration and response

The median ETV plasma concentration after 1 month of standard treatment was 0.384 ng/mL (IQR 0.297–0.569 ng/mL). Strikingly, this concentration showed a significant inverse correlation with HBV-DNA decrease after 3 months of treatment ($r = –0.624$; $P < 0.001$) (Fig. 1). This correlation was also confirmed when stratifying patients on the basis of viral genotypes: A ($r = –0.719$; $P = 0.003$); C ($r = –0.917$; $P = 0.007$); and D ($r = –0.760$; $P = 0.007$); genotypes B, E and F did not show the same correlation (probably for numerical reasons).

Furthermore, the ETV plasma concentration was significantly correlated with patient age ($r = 0.327$; $P = 0.039$) and baseline log HBV-DNA level ($r = –0.380$; $P = 0.015$). No significant correlation was observed between ETV levels after 1 month of treatment and HBsAg logarithmic decay after 3 months of treatment ($r = –0.217$; $P = 0.178$).

ETV median plasma concentrations after 2 months and 3 months were 0.414 ng/mL (IQR 0.293–0.484 ng/mL) and 0.490 ng/mL (IQR 0.384–0.614 ng/mL), showing no statistically significant differences with the concentrations at 1 month (*P*-values of 0.748 and 0.210, respectively).

ETV plasma concentrations at 2 months and 3 months of treatment were not significantly correlated with the HBV-DNA