Research Article

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Quantification of HBsAg in nucleos(t)ide-naïve patients treated for chronic hepatitis B with entecavir with or without tenofovir in the BE-LOW study

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Background & Aims: Serum hepatitis B surface antigen (HBsAg) levels may predict treatment response in chronic hepatitis B (CHB). We examined the association between changes in HBsAg levels and response to treatment in the BE-LOW study.

Methods: In this open-label, multicentre study, 379 nucleos(t)ide-naïve patients with hepatitis B e antigen (HBeAg)-positive or -negative CHB were randomized and treated with daily entecavir 0.5 mg alone (n = 182) or combined with tenofovir 300 mg (n = 197) for 100 weeks. HBsAg levels were quantified (Abbott Architect assay) at baseline and at Weeks 12, 48, and 96.

Results: Mean baseline HBsAg levels were comparable across subgroups by baseline alanine aminotransferase (ALT), genotype, age, and treatment type, but were higher in HBeAg-positive than in HBeAg-negative patients. Mean HBsAg changes from baseline at Weeks 12, 48, and 96 were more pronounced in HBeAg-positive than in HBeAg-negative patients, in patients with genotype A than in those with genotypes C or D, and in patients with elevated baseline ALT, but were similar between treatment groups and between patients of different age categories. Mean HBsAg changes over 96 weeks were also comparable in patients with or without HBV DNA <50 IU/ml at Week 96, but among patients that were HBeAg-positive at baseline, changes were greater for those with Week 96 HBeAg loss than for those without.

Conclusions: In this population of HBeAg-positive and HBeAg-negative, nucleos(t)ide-naïve patients, a greater HBsAg decline through 96 treatment weeks was observed in HBeAg-positive patients, especially in those who achieved subsequent HBeAg loss.

Keywords: Serum HBsAg quantitation; Entecavir; Tenofovir; Combination therapy.

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Abbreviations: CHB, chronic hepatitis B; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; peg-IFN, pegylated interferon; HBeAg, hepatitis B e antigen; ETV, entecavir; TDF, tenofovir disoproxil fumarate; ALT, alanine aminotransferase; ULN, upper limit of normal.

Introduction

Chronic hepatitis B (CHB) remains a significant public health burden, particularly in the Asia-Pacific region [1–3], accounting for approximately 1 million deaths annually worldwide. Since ongoing hepatitis B virus (HBV) replication is a key driver of CHB disease progression [4], the primary treatment goal is the sustained suppression of circulating HBV DNA to undetectable levels to achieve remission of liver disease and prevention of liver failure and hepatocellular carcinoma (HCC) [1–3]. Complete elimination of HBV is rare because the viral life cycle involves the formation of a stable intrahepatic reservoir of covalently closed circular DNA (cccDNA), which can result in virologic relapse following treatment cessation [5]. Hepatitis B surface antigen (HBsAg) is expressed from cccDNA-derived mRNA and released as part of infectious particles or non-infectious subvirion particles, the latter being produced independently of viral replication [6]. Hence, circulating HBsAg continues to be detectable in virologically suppressed patients, representing a serologically stable marker of viral persistence, the disappearance of which may be associated with the loss of the transcriptionally active cccDNA reservoir [7–9]. Hence, sustained elimination of HBsAg is the ultimate surrogate end point for successful treatment. This can be achieved by finite therapy with pegylated interferon (peg-IFN), but is only possible in a small minority of patients [10,11]. With nucleos(t)ide analogue antivirals, long-term treatment is often needed to maintain viral suppression, and even then HBsAg loss is infrequent [1–3].

The association between HBsAg and viral transcription has led to interest in circulating HBsAg levels as a quantitative prognostic marker of treatment response. A quantitative association between

circulating HBsAg and intrahepatic cccDNA has been observed in untreated hepatitis B e antigen (HBeAg)-positive patients, but, interestingly, not in HBeAg-negative patients [7]. Declines in serum HBsAg levels were shown to parallel those of intrahepatic cccDNA during adefovir dipivoxil therapy [8]. Quantitative HBsAg (qHBsAg) declines on peg-IFN-based treatment (with or without nucleos[t]ides) have been found to correlate with HBeAg seroconversion [12] and reductions in total intracellular HBV DNA [13] and intracellular cccDNA [14]. A decline in circulating qHBsAg is a strong predictor of sustained off-treatment response to peg-IFN therapy [15,16], but the role of HBsAg quantification in nucleos(t)ide treatment, which has a more limited effect on circulating levels, is less clear. It has been proposed that initial declines in qHBsAg levels may help predict the probability of, and time to, achievement of a serologic response and provide an indication of the necessary treatment duration. However, the results are inconsistent [17–23] and there are currently insufficient large-scale studies from which to draw firm conclusions [24].

Entecavir (ETV) and tenofovir disoproxil fumarate (TDF) are potent inhibitors of HBV polymerase and preferred first-line treatments for CHB treatment [1–3]. Both agents resulted in high rates of HBV DNA suppression and alanine aminotransferase (ALT) normalization in phase III studies of HBeAg-positive and -negative patients [25–27]. Maintenance of viral suppression in long-term studies has led to improvements in liver histology [28–30] and, with ETV, potential reduction in disease progression and incidence of HCC [31,32]. Both agents are associated with a low risk of resistance in nucleos(t)ide-naïve patients during long-term treatment [33,34] and with favourable safety profiles [35,36].

The BE-LOW study was designed to assess the efficacy and safety of ETV *vs.* ETV + TDF combination therapy in nucleos(t)ide analogue-naïve HBeAg-positive or HBeAg-negative, compensated CHB patients. The primary results of this study, which have been previously published [37], showed comparable proportions of patients in both arms achieving the primary end point of HBV DNA <50 IU/ml at Week 96 (83.2% *vs.* 76.4%; $p = 0.0882$), as well as HBeAg loss and seroconversion (ETV: 38.9% and 32.5%; ETV + TDF: 29.7% and 21.7%, respectively). The objective of this analysis was to examine the association between changes in qHBsAg levels and response to treatment in the BE-LOW study.

Patients and methods

Study design

This was a post-hoc analysis carried out on data from patients included in the BE-LOW study, a randomized, parallel, open-label, multicentre phase IIIb superiority study (clinicaltrials.gov NCT00410072), for which the full study design and primary efficacy results have been described previously [37]. Briefly, HBeAg-positive (n = 264) or HBeAg-negative (n = 115), nucleos(t)ide analogue-naïve CHB patients (⩾16 years old) with compensated liver function received once-daily ETV 0.5 mg (n = 182) or ETV 0.5 mg + TDF 300 mg (n = 197). The study duration was 100 weeks, with the primary efficacy time point at Week 96. The study was conducted in accordance with the principles of the Declaration of Helsinki and all local regulatory requirements. Institutional approval was obtained at all participating sites and written informed consent obtained prior to the initiation of study procedures.

Analyses

HBV DNA was assessed locally using the COBAS® TaqMan assay for the High Pure System (Roche Molecular Systems, Pleasanton, CA, USA) with a lower limit of quantitation of 29 IU/ml (approximately 169 copies/ml), and a lower limit of detection of 10 IU/ml (approximately 58 copies/ml). Serum HBsAg was quantitated retrospectively in frozen samples collected at baseline and at Weeks 12, 48, and 96. Diluted samples (1:150, 1:500, or 1:999) were analysed at a central laboratory, using the Abbott Architect assay (Abbott Laboratories, Abbott Park, IL, USA), with a dynamic range of 0.05–250 IU/ml. Serum qHBsAg levels were summarized across different baseline subgroups, and according to response to treatment (with or without virologic response [serum HBV DNA <50 IU/ml] or HBeAg loss at Week 96).

Statistical analysis

All data analyses are descriptive, due to the post-hoc nature of this analysis, which was not pre-specified. Except for the regression analyses described below, statistical inference was not conducted. Multivariate logistic regression analyses were carried out to identify factors associated with HBeAg loss and HBsAg loss. The choice of parameters included in the analyses (baseline factors: treatment group, HBsAg level, ALT level, HBV genotype [genotype A *vs.* non-A], HBV DNA level; Week 24 factors: HBV DNA and HBeAg reduction from baseline) was based on a previous study with ETV monotherapy [19].

Results

Study population

Baseline demographics and disease characteristics of the study population have been described previously [37]. There were some differences between HBeAg-positive and HBeAg-negative patients. In both treatment arms, HBeAg-negative patients were older than HBeAg-positive patients (mean age approximately 47 years *vs.* approximately 36 years; data not shown). Mean baseline HBV DNA was 7.5 $\log_{10}$ IU/ml overall, and about 2 $\log_{10}$ IU/ml higher among HBeAg-positive patients (around 8.1 $\log_{10}$ IU/ml) than among HBeAg-negative patients (6.1 $\log_{10}$ IU/ml). The distribution of HBV genotypes was fairly even overall, but differed between HBeAg-positive and HBeAg-negative patients, with a greater proportion of genotype D (47.0%) and a lower proportion of genotype A (9.6%) among HBeAg-negative patients than among HBeAg-positive patients (22.0% and 23.9%, respectively) [37].

Baseline qHBsAg levels

For this post-hoc analysis, qHBsAg levels at baseline were summarized across different subgroups. Overall, 360 patients (ETV: 178; ETV + TDF: 182) had baseline measurements for qHBsAg analysis; of these, 159 ETV-treated patients and 158 patients receiving ETV + TDF had paired baseline and Week 96 samples for qHBsAg analysis. Mean baseline qHBsAg levels were comparable across treatment arms (~4.0 $\log_{10}$ IU/ml), and across subgroups by baseline ALT, genotype, and age (Table 1; Supplementary Fig. 1). In both arms, mean baseline qHBsAg levels were around 0.8 $\log_{10}$ IU/ml higher among HBeAg-positive than among HBeAg-negative patients (Table 1), with baseline qHBsAg levels >5 $\log_{10}$ IU/ml recorded in 16% (39/249; among all patients with baseline qHBsAg measurement) of HBeAg-positive patients, and in none of the HBeAg-negative patients (data not shown). Mean baseline qHBsAg levels were also higher (by 0.5–0.8 $\log_{10}$ IU/ml) among patients who failed to achieve HBV DNA <50 IU/ml at Week 96 compared with those who did (Table 1); among those who did achieve a virologic response, >90% (260/284) had baseline qHBsAg levels ⩾2 $\log_{10}$ to ⩽5 $\log_{10}$ IU/ml, and <1% (2/284) had <2 $\log_{10}$ IU/ml, whereas only 8% (22/284) had >5 $\log_{10}$ IU/ml (data not shown).