Research Article

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Changes of HBsAg and interferon-inducible protein 10 serum levels in naive HBeAg-negative chronic hepatitis B patients under 4-year entecavir therapy

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Background & Aims: Serum HBsAg levels might represent an important predictor of sustained off-treatment response in HBeAg-negative chronic hepatitis B (CHB). We evaluated the changes of HBsAg and interferon-inducible protein 10 (IP10) serum levels in HBeAg-negative CHB patients treated with entecavir.

Methods: 114 patients received entecavir for a median of 4.3 years. HBsAg levels were determined at baseline, 6 and 12 months and every year thereafter until year-4. IP10 levels were measured at baseline and annually until year-4 in 76 patients.

Results: Virological remission rates were high (year-1: 94%, after year-2: 97–98%). Compared to baseline, HBsAg levels decreased by a median of 0.03, 0.13, 0.17, 0.22, and 0.32 $\log_{10}$ IU/ml at 6 months and 1, 2, 3, and 4 years, respectively ($p \leqslant 0.001$ for all comparisons). The proportions of patients with HBsAg decline of $\geqslant 0.5$ or $\geqslant 1$ $\log_{10}$ IU/ml were 9% or 6% at year-1 and 21% or 10% at the last visit. Median IP10 levels (pg/ml) did not change from baseline to year-1 or -2 (245 *vs.* 229 or 251), but increased at year-3 and -4 (275 and 323, p <0.030). HBsAg drop $\geqslant 0.5$ $\log_{10}$ was associated with baseline IP10 or IP10 >350 pg/ml ($p \leqslant 0.002$). HBsAg loss occurred in 4/114 (3.5%) patients or in 1/2, 3/21, and 0/91 patients with baseline HBsAg <100, 100–1000 and >1000 IU/ml, respectively (p <0.001).

Conclusions: In HBeAg-negative CHB patients, 4-year entecavir therapy decreases serum HBsAg levels, but the rate of decline is rather slow. Serum IP10 levels represent a promising predictor of HBsAg decline in this setting.

Keywords: Hepatitis B; Entecavir; HBsAg; Interferon-inducible protein 10.
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Abbreviations: HBeAg, hepatitis B e antigen; NA(s), nucleos(t)ide analogue(s); HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; anti-HBe, antibody against HBeAg; ALT, alanine aminotransferase; ULN, upper limit of normal; PCR, polymerase chain reaction; LSM, liver stiffness measurement; IQR, interquartile range; SD, standard deviation.

Introduction

Although the treatment of chronic hepatitis B (CHB) has substantially improved over the last 15 years, it remains suboptimal particularly for HBeAg-negative patients [1–3]. Pegylated interferon-alfa (PegIFNα) induces sustained off-treatment responses in ⩽25% of HBeAg-negative patients, while the first-line nucleos(t)ide analogues (NAs) achieve virological on-therapy remission in almost all HBeAg-negative patients, but they should be given for long periods, perhaps indefinitely [1–4]. There have been efforts to identify reliable predictors of safe discontinuation of NAs in HBeAg-negative CHB but without success so far [4].

HBsAg represents the only serological marker in HBeAg-negative CHB [5]. The recent development of rapid, easy to use and reliable quantitative assays for serum HBsAg levels has revived the interest in this marker, particularly for PegIFNα treatment [6]. However, since HBsAg clearance is the optimal therapeutic end-point and a safe marker of NA discontinuation even in patients with HBeAg-negative CHB [3], a decline of HBsAg levels might be an important predictor of off-NA remission [6,7]. Although there are recent studies evaluating the changes of HBsAg levels under NAs, most of them are small and include both HBeAg-positive and HBeAg-negative patients mostly treated with NAs of low genetic barrier [8–11]. In addition, serum levels of interferon-inducible protein 10 (IP10), an interferon–γ inducible protein of 10 kDa, were recently suggested to represent a predictive marker of HBsAg loss during NA therapy [12], but they have not been adequately assessed in this setting.

We evaluated the changes of serum HBsAg and IP10 levels in a relatively large cohort of HBeAg-negative CHB patients treated with entecavir, which was the first high genetic barrier NA introduced in daily clinical practice.

Patients and methods

Patient population

All adult, NA naive patients with HBeAg-negative CHB seen at our liver clinics between June 2010 and June 2011 were included in this retrospective-prospective study if they had (a) an established diagnosis of HBeAg-negative CHB before therapy based on positive HBsAg, negative HBeAg and positive antibody against HBeAg (anti-HBe) for ⩾6 months, increased alanine aminotransferase (ALT) activity [higher than the upper limit of normal (ULN): 40 IU/L] on ⩾2 separate monthly determinations within the last 6 months, HBV DNA >2000 IU/ml and exclusion of other causes of liver injury and (b) started entecavir ⩾6 months before the final study approval (December 2009). Patients who had received NAs in the past, did not have stored serum samples taken before and at least annually after entecavir onset, did not complete ⩾12 months of entecavir as well as patients with decompensated liver disease, hepatocellular carcinoma or any malignancy, liver transplantation or co-infection with hepatitis D or C or human immunodeficiency virus were excluded.

The study was approved by the ethics committees of the two participating hospitals and the Hellenic Drug Organization. All patients gave written informed consent.

Follow-up

All patients received entecavir at standard dosage (0.5 mg once daily). Epidemiological and anthropometrical characteristics, abdominal ultrasound, routine laboratory tests and serological markers were obtained before treatment according to standard clinical practice. Routine laboratory tests were repeated every 3 months during the first year and every 6 months thereafter. HBV DNA was detected before treatment and every six months during treatment. Initial virological response was defined as undetectable HBV DNA within the first year. Virological breakthrough was defined as HBV DNA reappearance after undetectability or ⩾1 $\log_{10}$ increase compared to treatment nadir. Maintained virological remission was defined as HBV DNA undetectability even beyond the first year and maintenance of such a response until the end of follow-up. This study ended at year-4, which was the follow-up period for the majority of cases.

Methods

Commercially available assays were used for routine laboratory tests and serological markers. HBV DNA was detected using a sensitive in-house real-time polymerase chain reaction (PCR) assay, as described previously (sensitivity: 45 IU/ml) [19].

HBsAg levels were retrospectively determined in stored serum samples at baseline and at 6 months and 1, 2, 3, and 4 years of therapy by a chemiluminescent microparticle immunoassay with a range of 0.05–250 IU/ml on the Architect analyzer (Abbott Laboratories, Abbott Park, IL). Sera were initially tested after a manual 1:100 dilution and, if needed, undiluted or further diluted 1:1000. According to the manufacturer datasheet, the inter- and intra-assay variability is approximately 10% (range 6.7–8.8%). This assay reproducibility in our lab has been excellent with a median $\log_{10}$ HBsAg coefficient of variation of 2.3%.

Serum IP10 levels were determined retrospectively in stored serum samples taken from 76 patients at baseline and 1, 2, 3, and 4 years of therapy. IP10 was measured by a solid phase sandwich ELISA (BioVendor, Karasek, Chech Republic) with a measuring range of 5.7–200 pg/ml. Sera were tested after a 1:2 dilution and further diluted 1:4 if IP10 levels were found above the upper limit of the test.

Liver biopsies were performed within the last 12 months before treatment in 45 patients. Histological changes were evaluated according to Ishak's classification [13]. After June 2007, 52 patients underwent transient elastography (FibroScan®, Echosens, France) before therapy. Liver stiffness measurements (LSM) (in kPa) by transient elastography were considered to be reliable if 10 successful measurements were obtained, with a success rate >60% and a ratio of interquartile range (IQR) to mean stiffness <30%. Based on histological and elastography findings, cirrhosis was diagnosed in patients with staging score 5–6 and/or LSM >12.5 kPa.

Statistical analysis

All data were analyzed using the statistical package SPSS Statistics (SPSS Inc, an IBM Company). Quantitative variables were expressed as mean values ± standard deviation (range) or median (range) values. Statistical analysis was performed using *t* test or Mann-Whitney test, paired *t* test or Wilcoxon matched paired test, corrected chi-square test or two-tailed Fisher's exact test and Spearman correlation, when appropriate. Survival curves were used for the estimation of responses over time. Cox regression analysis was used to assess predictors of HBsAg decline ⩾0.5 $\log_{10}$ IU/ml. The estimations of treatment duration required to achieve 1 $\log_{10}$ HBsAg decline or HBsAg clearance were based on HBsAg levels decline curves assessed by a linear mixed effects model with random intercept and slope, as previously applied [11]. A two-tailed *p* value of <0.05 was considered to be statistically significant.

Results

Main patient characteristics

In total, 128 NA naive patients with compensated HBeAg-negative CHB seen between June 2010 and June 2011 had started entecavir monotherapy before December 2009. Fourteen were excluded because of unavailability of stored serum samples (n = 12) or follow-up <12 months (n = 2). The main pre-treatment characteristics of the 114 included patients are presented in Table 1. There was no significant difference in the characteristics between the 114 included and the 14 excluded patients (data not shown).

The virological response rates over the first 4 years are presented in Fig. 1. Initial virological response was achieved in 94% (107/114) and maintained virological remission in 98% (112/114) of patients. Two patients had temporary virological breakthroughs associated with poor drug compliance.

Table 1. Baseline characteristics of 114 patients with HBeAg-negative chronic hepatitis B.

Age, yr	52 ± 14 (19-78)
Male sex, n (%)	71 (62.3)
Origin, n (%)	
Greece	85 (74.6)
Albania	22 (19.3)
Other	7 (6.1)
Body mass index, kg/m²	26.3 ± 4.0 (18.2-37.5)
ALT, IU/L	72 (21-1270)
ALT elevation, n (%)	
<ULN	21 (18.4)
ULN-2 x ULN	43 (37.7)
>2 x ULN	50 (43.9)
Serum HBsAg, $\log_{10}$ IU/ml	3.51 (1.76-4.95)
Patients with HBsAg, n (%)	
<100 IU/ml	2 (1.8)
100-1000 IU/ml	21 (18.4)
>1000 IU/ml	91 (79.8)
Serum HBV DNA, $\log_{10}$ IU/ml	5.3 (3.3-9.4)
Liver biopsy, n (%)	45 (39.5)
Grading score	7.1 ± 2.7 (4-14)
Staging score	2.8 ± 1.2 (1-5)
Transient elastography, n (%)	52 (45.6)
Liver stiffness, kPa	11.4 ± 7.8 (3.4-44.3)
Cirrhosis, n/N (%)	17/89 (19.1)
PegIFNα in the past, n (%)	20 (17.5)
Follow-up, yr	4.3 (1.1-6.3)

Quantitative variables are expressed as mean values ± standard deviation (range) or median values (range).