Research Article

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Long-term hepatitis B surface antigen (HBsAg) kinetics during nucleoside/nucleotide analogue therapy: Finite treatment duration unlikely

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Background & Aims: Information regarding long-term HBsAg kinetics during treatment with nucleoside/nucleotide analogues is limited. The aim of the present study was to assess whether finite nucleoside/nucleotide analogue treatment duration could be envisaged during the patient's lifetime.

Methods: Patients with chronic hepatitis B receiving different schedules of nucleoside/nucleotide analogues were followed for a median duration of 102 months, i.e., 8.5 years (interquartile range: 88–119 months). Long-term HBV DNA and HBsAg level kinetics were modeled in order to estimate time to clear HBsAg during therapy in patients with undetectable HBV DNA.

Results: Antiviral therapy was associated with a slow but consistent reduction in the level of HBsAg in most of the patients. Three patterns of HBsAg level declines were identified: decline during both the detectable and undetectable HBV DNA phases; decline during the HBV DNA detectable period only; decline during the HBV DNA undetectable period only. The mean HBsAg titer at the time when HBV DNA became undetectable was 3.29 ± 0.49 Log_{10} international units (IU)/ml, and the mean slope was -0.007 ± 0.007 Log_{10} IU/month, i.e., an average decline of 0.084 Log_{10} IU/year. The corresponding calculated median number of years needed to clear HBsAg was 52.2 years (interquartile range: 30.8–142.7).

Conclusions: This study, based on the very long-term follow-up of patients with chronic hepatitis B treated with potent nucleoside/nucleotide analogues, shows that HBsAg clearance is unlikely to occur during the patient's lifetime, even if HBV replication is well controlled. Thus, lifetime therapy is required in the vast majority of HBV-infected patients.

Introduction

Recent developments in the antiviral treatment of chronic hepatitis B virus (HBV) infection have emphasized the need for biomarkers that are predictive of treatment outcomes and can be used to tailor therapy to the individual patient. In chronic HBV carriers, hepatitis B surface antigen (HBsAg) is produced as a result of translation of messenger RNAs generated from transcriptionally active cccDNA or integrated HBV DNA sequences in the host genome. HBsAg is present in the envelope of infectious HBV virions and in non-infectious spheres and tubules. The latter exceed infectious virions when replication is active; they remain produced in large amounts when replication is controlled, either spontaneously (inactive carriers) or by antiviral therapy [1–4].

A number of recent studies have demonstrated the clinical utility of HBsAg quantification in monitoring HBV therapy [1,5–14]. Indeed, early on-treatment serum HBsAg levels predict the sustained post-treatment response to therapy and the eventual subsequent HBsAg clearance in patients with both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative chronic hepatitis B treated with a finite duration of pegylated interferon (IFN)-α [1,5–9]. HBsAg clearance, followed or not by HBs seroconversion (appearance of anti-HBs antibodies) characterizes a sustained remission of HBV infection.

Standardized quantitative HBsAg level assays are available. Three commercial assays, the HBsAg assay on Architect® device (Abbott Diagnostics, Chicago, Illinois), the HBsAg II Quant assay on Elecsys® or Cobas® e devices (Roche Diagnostics GmbH, Mannheim, Germany), and the Liaison®XL HBsAg Quant assay on Liaison®XL device (DiaSorin, Saluggia, Italy) are approved in the European Union; they are available for research use only in

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Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; cccDNA, covalently closed circular DNA; HBeAg, hepatitis B e antigen; IFN, interferon; IU, international unit; HDV, hepatitis D virus; PCR, polymerase chain reaction; IQR, interquartile range.

the United States. These tests are inexpensive and easy-to-use, and high throughput is possible with automated platforms [15,16]. Thus, HBsAg quantification can be easily used to monitor antiviral therapy in patients with chronic HBV infection.

Treatment of chronic hepatitis B with nucleoside or nucleotide analogues is aimed at reducing virus production by inhibiting the reverse transcriptase function of the HBV DNA polymerase, the enzyme responsible for viral replication. Nucleoside/nucleotide analogues do not exert a direct effect on HBsAg transcription and translation. Recent liver society guidelines recommended the use of entecavir or tenofovir as first-line therapy in patients with chronic hepatitis B and an indication for nucleoside/nucleotide analogues, because both drugs potently inhibit the HBV reverse transcriptase and have a high barrier to resistance. Entecavir and tenofovir have been shown to efficiently maintain suppression of HBV DNA levels for prolonged periods of time in the vast majority of treated patients [17–19]. As a result, most patients who started therapy with other drugs currently receive entecavir and/or tenofovir as part of their treatment regimen in areas where these drugs are available and reimbursed.

Information regarding HBsAg kinetics during treatment with nucleoside/nucleotide analogues is limited [5,10–14,18,20–25]. These studies indicate nucleoside/nucleotide analogue therapy results in lesser overall declines in serum HBsAg levels and HBsAg clearance appears to be less frequent than in patients receiving pegylated IFN-α-based therapy. However, this could be biased by the fact that pegylated IFN-α is generally used in subsets of patients with a greater likelihood to clear HBsAg, e.g., patients with low HBV DNA and high alanine aminotransferase levels.

The aim of the present study was to assess whether finite nucleoside/nucleotide analogue treatment duration could be envisaged during the patient's lifetime. For this, we assessed the long-term kinetics of serum HBsAg levels in patients with chronic hepatitis B treated with various successive schedules of nucleoside/nucleotide analogues and who maintained undetectable HBV DNA in the long term, and evaluated the clinical interest of HBsAg level quantification to tailor treatment duration.

Patients and methods

Patients

The study was a longitudinal analysis of HBsAg levels in prospectively collected serum samples from 30 patients with histologically proven chronic hepatitis B, treated with different nucleoside/nucleotide analogue schedules, according to drug approvals and outcomes on therapy, between December 1996 and December 2010, in the Department of Hepatology and Gastroenterology of our institution. All patients were positive for HBsAg and negative for anti-HBs antibodies at the beginning of the study. The selection criteria were a follow-up on treatment of at least 4 years and availability of stored sera for quantification of HBsAg levels. All patients followed in our institution who met these criteria were included; they were representative of HBV-infected patients seen in the area, as shown by their genotype distribution (Table 1). A total of 604 serial samples (mean: 20.1 ± 7.4 per patient; range: 10–42) collected from April 2000 to December 2010 were available (no samples available prior to 2000). The study followed the principles of Good Clinical Practice and was approved by the local ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale). All patients gave written informed consent. The drugs were given once daily at doses of 100 mg for lamivudine, 10 mg for adefovir dipivoxil, 0.5 mg or 1.0 mg for entecavir, and 300 mg for tenofovir disoproxil fumarate.

Laboratory measurements

Serum HBV DNA levels were measured in each available sample by means of a real-time PCR assay (COBAS Ampliprep®/COBAS TaqMan®, Roche Molecular Systems, Pleasanton, California) [26]. Results were expressed in international units per milliliter (IU/ml). The lower limit of detection of the assay is 20 IU/ml.

Serum HBsAg levels were quantified by means of the Architect® HBsAg assay after 1:100 dilution of the sera. The dynamic range of quantification of this assay is 0.05–250.0 IU/ml (−1.3–2.4 Log_{10} IU/ml). Samples with HBsAg levels <0.05 IU/ml at 1:100 dilution were retested undiluted, while those with HBsAg >250.0 IU/ml at 1:100 dilution were retested at a final dilution of 1:999, according to the manufacturer's instructions.

The HBe status was assessed with commercial enzyme immunoassays (VIDAS™ HBe and VIDAS™ Anti-HBe, Biomérieux, Marcy-l'Etoile, France). Anti-hepatitis D virus (HDV) antibodies were detected with an enzyme immunoassay (ETI-AB-DELTAK-2, Bio-Rad Laboratories, Hercules, California). Basal core promoter and precore mutations were sought by means of a line probe assay after nested PCR amplification of the corresponding genomic region (INNO-LiPA HBV PreCore, Innogenetics, Gent, Belgium), according to the manufacturer's instructions.

The HBV genotype was determined in all patients by directly sequencing a portion of the overlapping genes encoding HBsAg and the B and C subdomains of the HBV reverse transcriptase. Sequence analysis was followed by phylogenetic analysis. Briefly, after extraction of viral DNA from 200 µl of serum using the QIAamp MinElute Virus Vacuum Kit (Qiagen GmbH, Hilden, Germany), a hemi-nested polymerase chain reaction (PCR) was used to amplify a 492-bp fragment with primers *POL-1*, *POL-2* and *HBPr-94*, as previously described [27–29]. PCR products were sequenced by means of the Big-Dye Terminator v3.1 sequencing kit on the ABI 3100 sequencer (Applied Biosystems, Foster City, California), according to the manufacturer's protocol. Phylogenetic analyses were performed using different prototype HBV genotype A–H sequences, with software from the Phylogeny Inference Package (PHYLIP) version 3.65.

Statistical analysis

Statistical analysis was performed with Stata® 10.0 (StataCorp LP, College Station, Texas). We assumed that the HBsAg titer follows a Log-normal distribution. Patient-specific slopes of HBsAg decline were estimated using linear regression and used to predict time to undetectable HBsAg whenever the decline was statistically significant, using the first sample with undetectable HBV DNA as a starting point. The sample distribution of times to undetectable HBsAg was described using the median and dispersion has been characterized by the first and third quartiles (interquantile range [IQR]). In addition, the population-level mean and standard deviation were estimated using a mixed linear model with random coefficients and similarly used to estimate the time to undetectable HBsAg.

Results

Characteristics of the study patients

Table 1 shows the individual demographic, virological and histological data of the patients. Twenty-four men and 6 women were enrolled in the long-term longitudinal study of HBsAg levels. The mean age of the patients was 55.6 ± 10.1 years (range: 30.6–88.1); 16 of them were Caucasian, 6 were Asian, and 8 were African. Eighteen patients (60.0%) were HBeAg-negative. Among the 12 patients who were HBeAg-positive before starting therapy, 6 were still HBeAg-positive at the beginning of follow-up in this study; 3 of them seroconverted during the study period and simultaneously became HBV DNA-negative.

The HBV genotype could be identified in 28 of the 30 patients. Nine patients were infected with genotype A, 6 with genotype B, 1 with genotype C, 4 with genotype D, 5 with genotype E, 1 with genotype F, and 2 with genotype G. In the remaining patients, no sample with an HBV DNA level >2 Log_{10} was available during follow-up for HBV genotype determination in one case and the genotype was indeterminate in spite of efficient PCR amplification in the other case. Twenty-five of the 30 patients (83.3%) had significant fibrosis (METAVIR grade ⩾F2) and 13 patients had compensated cirrhosis (F4). None of them was co-infected with HDV (Table 1).