Nucleos(t)ide Analogues Only Induce Temporary Hepatitis B e Antigen Seroconversion in Most Patients With Chronic Hepatitis B

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BACKGROUND & AIMS: Inconsistencies in results and guideline recommendations regarding the durability of nucleos(t)ide analogue-induced hepatitis B e antigen (HBeAg) seroconversion require clarification. We studied the long-term durability of nucleos(t)ide analogue-induced HBeAg seroconversion in patients with chronic hepatitis B virus (HBV) infection. **METHODS:** We performed a single-center cohort study of 132 HBeAg-positive patients who had received nucleos(t)ide analogue therapy. **RESULTS:** During a median treatment duration of 26 months (range, 16–43 mo), HBeAg seroconversion occurred in 46 of 132 subjects (35%). Forty-two subjects (91%) had follow-up evaluation after HBeAg seroconversion. During a median follow-up period of 59 months (range, 28–103 mo) after HBeAg seroconversion, 13 of 42 patients (31%) showed a durable remission (defined as HBeAg negative and HBV-DNA level <10,000 copies/mL). Overall, 33 of 42 subjects (79%) continued therapy after HBeAg seroconversion; of these, 22 (67%) showed serologic and/or virologic recurrence. Nine of 42 subjects (21%) discontinued therapy after HBeAg seroconversion and at least 6 months of consolidation therapy. Only 2 patients showed a durable response in the absence of therapy. Disease recurrence in patients who continued therapy after HBeAg seroconversion was preceded by the development of resistance (80% of these patients); resistance only occurred in subjects given lamivudine monotherapy. In contrast, recurrence after treatment discontinuation or noncompliance was observed in all patients given nucleos(t)ide analogues. **CONCLUSIONS: Induction of HBeAg seroconversion by nucleos(t)ide analogues is temporary in most patients with chronic HBV infection. Long-term continuation of nucleos(t)ide analogue treatment, irrespective of the occurrence of HBeAg seroconversion, appears to be necessary.**

Keywords: Durability; Antiviral Therapy; Discontinuation; Sustained Response.

With approximately 400 million persons infected worldwide, chronic hepatitis B virus (HBV) infection is a major challenge in medical health care. The current treatment aims to prevent the development of cirrhosis and hepatocellular carcinoma.[1] Because these end points can be assessed only after decades of infection, short-term surrogate outcomes are used to assess the effect of therapeutic regimens. In hepatitis B e antigen (HBeAg)-positive patients, an important end point is HBeAg loss or seroconversion because it usually is associated with sustained remission and a low risk for the development of cirrhosis and hepatocellular carcinoma.[2–7]

Nucleos(t)ide analogues interfere with the elongation of viral DNA chains through competitive inhibition with the viral polymerase, and are potent inhibitors of viral replication.[8] In general, HBeAg seroconversion rates are approximately 20% after 1 year of nucleos(t)ide analogue treatment, and these rates increase with prolonged therapy. International guidelines on the therapy of hepatitis B suggest that finite duration of treatment with nucleos(t)ide analogues is a reasonable option, and recommend that treatment may be stopped after HBeAg seroconversion and an additional 6–12 months of consolidation therapy.[9,10]

However, the long-term durability of HBeAg seroconversion induced by nucleos(t)ide analogues is controversial because several studies have reported contradictory results. Although some investigators have reported nucleos(t)ide analogue-induced HBeAg seroconversion to be durable in up to 90%, others have reported relapse rates as high as 70%.[11–13] Studies investigating durable response to newer agents such as entecavir and tenofovir are still scarce. Furthermore, off-treatment response data for nucleos(t)ide analogues are available for relatively short periods only, whereas interferon-induced response has been documented to be durable for years with increasing numbers of hepatitis B surface antigen (HBsAg) seroconversion.[14–16]

Clearly, inconsistencies in results and guideline recommendations regarding the durability of nucleos(t)ide an-

Abbreviations used in this paper: ADV, adefovir; anti-HBe, antibody against hepatitis B e antigen; anti-HBs, antibody against hepatitis B surface antigen; LAM, lamivudine.

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alogue-induced HBeAg seroconversion require clarification. We therefore studied the long-term durability of nucleos(t)ide analogue-induced HBeAg seroconversion.

Materials and Methods

Study Population

All adult patients with chronic HBV infection (HBsAg-positivity for at least 6 months), referred to the Erasmus Medical Center between 1996 and 2009, who were treated with nucleos(t)ide analogue therapy for at least 6 months, and who tested HBeAg positive, antibody against HBeAg (anti-HBe) negative at the start of treatment, were eligible. Major exclusion criteria were as follows: co-infection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus, and treatment with (pegylated) interferon for less than 6 months before the start of nucleos(t)ide analogue treatment.

Follow-Up Evaluation

All subjects were monitored at least every 3–6 months. Biochemical (serum alanine aminotransferase [ALT]) and virologic parameters (quantitative HBV DNA, HBeAg, and anti-HBe levels) were assessed at every visit. HBV genotype was determined in available serum samples. HBsAg and antibody against HBsAg (anti-HBs) values were determined in all patients who were HBeAg-negative with HBV-DNA levels lower than 1000 copies/mL at the last follow-up evaluation. A genotypic analysis was performed in case of virologic breakthrough, defined as an increase in serum HBV-DNA level greater than 1 $\log_{10}$ (10-fold) above nadir after initial virologic response. Patients were asked to confirm their adherence to their treatment regimen at all outpatient clinic visits. Patients were defined as noncompliant if unexpected virologic rebounds were observed, and the treating physician clearly judged the patient to be nonadherent.

End Points

HBeAg seroconversion was defined as loss of HBeAg with concurrent appearance of anti-HBe. Serologic recurrence was defined as reappearance of HBeAg confirmed by HBeAg positivity in a consecutive sample. Because a viral load of more than 10,000 copies/mL is associated with progression of liver disease,[17,18] virologic recurrence was defined as an increase of HBV-DNA level to greater than 10,000 copies/mL after HBeAg seroconversion with previously HBV-DNA levels less than 10,000 copies/mL, confirmed in a consecutive sample.

Laboratory Testing

Serum ALT level was measured using automated techniques. The upper limit of normal was 40 IU/L for male and 30 IU/L for female subjects. HBsAg, HBeAg, and anti-HBe were determined using commercially available enzyme immunoassays. Serum HBV-DNA levels were measured using a previously described quantitative real-time polymerase chain reaction developed in-house.[19,20] Currently, this assay is multiplexed without compromising the lower limit of detection (373 copies/mL) with an internal control (Phocid Herpes Virus Type 1) to control the process from DNA isolation through polymerase chain reaction.[21] HBV DNA was extracted from serum samples using the MagnaPureLC (Roche Applied Science, Almere, The Netherlands) as described before.[19] HBV genotypes were assessed by sequence alignment of the overlapping HBsAg with HBV sequences derived from GenBank. The presence of HBV polymerase gene mutations was determined using InnoLiPA DR2 and DR3 line probe assay (Innogenetics, Gent, Belgium).

Statistical Analysis

Continuous variables are expressed as means (±standard deviation) or median (interquartile range) where appropriate. Follow-up times were calculated from the date of nucleos(t)ide analogue treatment initiation to the date of event or censorship. Cumulative probabilities of different end points were estimated by Kaplan–Meier analysis. Survival analysis with Cox regression model was used to analyze which of the following baseline factors were associated with HBeAg seroconversion and with serologic and/or virologic recurrence after development of HBeAg seroconversion: age, sex, race, body mass index, HBV genotype, time to HBeAg seroconversion, viral load at initiation of nucleos(t)ide analogue therapy and at HBeAg seroconversion, ALT level at initiation of nucleos(t)ide analogue therapy and at HBeAg seroconversion, presence of cirrhosis, and treatment regimen (lamivudine [LAM] monotherapy vs other treatment regimens).

Results

Baseline characteristics of all patients are shown in Table 1. A total of 132 patients with chronic HBV infection treated with nucleos(t)ide analogues in our hospital were included for analysis. Overall, 67 patients were treated with LAM monotherapy, 33 with adefovir (ADV), 22 with entecavir, 6 with tenofovir, 2 with ADV–LAM combination therapy, and 2 with tenofovir-LAM. A total of 117 (89%) patients were nucleos(t)ide analogue treatment-naive, whereas 15 (11%) subjects had been treated previously with nucleos(t)ide analogues. The median duration of therapy was 26 months (range, 16–43 mo). Of all patients, 100 (76%) subjects were men and the mean age was 38 ± 15 years. The most common HBV genotypes were genotypes A (35%) and D (26%).

HBeAg Seroconversion

HBeAg loss occurred in 55 of 132 (42%) subjects, of whom 46 (84%) patients seroconverted to anti-HBe during the study period. The cumulative probabilities of achieving HBeAg seroconversion after 1, 2, and 4 years of treatment were 25%, 32%, and 44%, respectively (Figure 1). The median duration of therapy until HBeAg sero-