# PANCREAS, BILIARY TRACT, AND LIVER

## Reduction of Hepatitis B Surface Antigen and Covalently Closed Circular DNA by Nucleos(t)ide Analogues of Different Potency

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**BACKGROUND & AIMS:** **Few studies have investigated the effects of different nucleos(t)ide analogues against hepatitis B virus (HBV) on levels of covalently closed circular DNA (cccDNA) and hepatitis B surface antigen (HBsAg) in patients. We measured the magnitude of reduction of cccDNA and HBsAg by nucleos(t)ide analogue therapy and assessed the correlation between their reductions.**

**METHODS:** **We recruited 124 patients who were treated with 1 of the 5 nucleos(t)ide analogues (lamivudine, adefovir, entecavir, telbivudine, or clevudine). All patients had undergone liver biopsy when treatment began (baseline) and 1 year later. The cccDNA and HBsAg levels were measured by real-time polymerase chain reaction and the Elecsys II HBsAg Assay, respectively.**

**RESULTS:** **After 1 year of treatment, HBV in 7 patients had become resistant to the nucleos(t)ide analogue. The remaining 117 patients had an average reduction of approximately 0.2 $\log_{10}$ IU/mL in HBsAg, 5 $\log_{10}$ IU/mL in serum level of HBV DNA, 2 $\log_{10}$ copies/cell in intrahepatic total HBV DNA, and 1 $\log_{10}$ copies/cell in cccDNA. Although 88 of 117 patients (75%) had undetectable serum levels of HBV DNA (<12 IU/mL), all had detectable levels of HBsAg, and only 5 (4%) had undetectable levels of cccDNA. On treatment with nucleos(t)ide analogues, patients with greater reductions in levels of cccDNA had greater reductions in HBsAg, but these reductions did not reach statistically significant correlations.**

**CONCLUSIONS:** **Although nucleos(t)ide analogues potently reduced serum levels of HBV DNA, the reduction of HBsAg and cccDNA was small. In short-term therapy, the magnitude of HBsAg reduction does not correlate with that of cccDNA reduction.**

*Keywords:* Antiviral Therapy; Viral Hepatitis; Response to Therapy; Prognosis.

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Chronic hepatitis B (CHB) infection affects about 400 million people worldwide.[1] Chronicity of infection is maintained by the persistence of hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) in hepatocytes and manifested by the secretion of HBV virions into the peripheral blood. cccDNA is the template for the transcription of all HBV mRNA and the pregenomic RNA, from which the HBV DNA genome and other HBV DNA intermediates originate.

Recently, there has been a great advance in the treatment of CHB, owing to the development of pegylated interferon and nucleos(t)ide analogues (NAs). Five NAs (lamivudine, adefovir, entecavir, telbivudine, and tenofovir) are approved for the treatment of CHB. These NAs typically decrease serum HBV DNA by 4–6 $\log_{10}$ copies/mL in the first year of therapy.[2–8] Because cccDNA is not a direct target of NAs, on treatment withdrawal, HBV DNA replication can resume from the residual cccDNA, resulting in HBV DNA rebound.[9]

Although the effects of NAs on serum HBV DNA levels are well documented, studies on their effects on intrahepatic HBV DNA, especially cccDNA levels, are relatively scanty. Several small-scale studies have demonstrated that 1 year of antiviral therapy reduced cccDNA by about 1 $\log_{10}$ copies/cell.[10–12] Because of the requirement of repeat liver biopsies, large-scale studies are limited.

*Abbreviations used in this paper:* cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NA, nucleos(t)ide analogue; qHBsAg, quantitative hepatitis B surface antigen.


1542-3565/$36.00
http://dx.doi.org/10.1016/j.cgh.2013.01.026

Recently, there has been an increasing interest in the use of quantitative hepatitis B surface antigen (qHBsAg) as an additional viral marker for the monitoring of disease progress and response to pegylated interferon therapy.[13–15] However, the correlation between qHBsAg and serum and intrahepatic HBV DNA levels varies between studies.[12,13,16,17] Marked reduction of qHBsAg has been observed in patients with pegylated interferon therapy.[18–21] Comparatively, the effect of NAs on HBsAg reduction and its correlation with cccDNA reduction are less well studied.

In the present study with a large series of patients with paired liver biopsies, we aimed to investigate the effects of 1-year therapy of several NAs, namely lamivudine, adefovir, entecavir, telbivudine, and clevudine, on qHBsAg, intrahepatic total HBV DNA, and cccDNA levels. We also investigated whether the magnitude of reduction of qHBsAg can reflect that of cccDNA levels during NA therapy.

## Methods

### *Patients*

We recruited 124 CHB patients from our center who took part in 3 international phase III, randomized, double-blind, multicenter trials, including (1) 36 patients from the BEHoLD trials, sponsored by Bristol-Myers Squibb, evaluating the efficacy of entecavir vs lamivudine[5,6]; (2) 64 patients from the GLOBE trial, sponsored by Idenix Pharmaceuticals and Novartis Pharmaceuticals, evaluating the efficacy of telbivudine vs lamivudine[7]; and (3) 24 patients from the QUASH trial, sponsored by Pharmasset, evaluating the efficacy of clevudine vs adefovir. All patients had baseline and 1-year biopsies performed. All liver biopsies were treated with the Allprotect Tissue Reagents (Qiagen, Hilden, Germany) and subsequently stored at −80°C. This study was approved by the Institution Review Board, Queen Mary Hospital, University of Hong Kong.

### *Measurement of Quantitative Hepatitis B Surface Antigen and Serum Hepatitis B Virus DNA*

The qHBsAg level was measured by using the Elecsys II HBsAg Assay (Roche Diagnostics, Branchburg, NJ), with an automatic on-board dilution with a dynamic range of 0.05–52,000 IU/mL. Serum HBV DNA was measured by the COBAS TaqMan HBV Monitor Test (Roche Diagnostics), with a dynamic range of 12–$1.1 \times 10^8$ IU/mL. Samples with qHBsAg >52,000 IU/mL or HBV DNA >$1.1 \times 10^8$ IU/mL were manually diluted with diluents or negative human plasma supplied by the manufacturer and retested to assess the accurate levels.

### *Measurement of Intrahepatic Hepatitis B Virus DNA and Covalently Closed Circular DNA*

Intrahepatic DNA was isolated by the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Intrahepatic total HBV DNA, cccDNA, and human genomic DNA were measured by real-time polymerase chain reaction as previously described.[11,22] Taking into account the tissue size used, we determined that the lower limits of detection of intrahepatic total HBV DNA and cccDNA quantification were $2 \times 10^{-4}$ and $5 \times 10^{-4}$ copies/cell, respectively.

### *Hepatitis B Virus Drug Resistance Detection*

The INNO-LiPA HBV DR line probe assays (Innogenetics NV, Gent, Belgium) were used to determine HBV drug-resistant mutations in patients with detectable HBV DNA at year 1.

### *Statistical Analyses*

Statistical analyses were performed by using the Statistical Program for Social Sciences (SPSS 18.0; SPSS Inc, Chicago, IL). The Mann–Whitney *U* test was used to test continuous variables with skewed distribution, and the Student *t* test was used to test normally distributed variables or logarithmic-transformed skewed variables. Related variables were compared by using the Wilcoxon signed rank test or paired-sample *t* test. Categorical variables were tested by the $\chi^2$ test, with continuity correction when appropriate, or the Fisher exact test when appropriate. Bivariate correlation analyses were used to test the correlation between 2 variables, with *P* values obtained on the basis of the Fisher transformation on the Pearson correlation coefficients. Serum HBV DNA, intrahepatic total HBV DNA, and cccDNA levels below the lower limit of detection of the assays were recorded as 12 IU/mL, $2 \times 10^{-4}$ copies/cell, and $5 \times 10^{-4}$ copies/cell, respectively, for the purpose of statistical calculation. Statistical significance was defined by $P < .05$.

## Results

### *Patient Characteristics at Baseline*

This study included 45 lamivudine-treated patients, 8 adefovir-treated patients, 20 entecavir-treated patients, 35 telbivudine-treated patients, and 16 clevudine-treated patients. On the basis of the antiviral potency of the NAs on the suppression of serum HBV DNA levels, the NAs were divided into 2 groups: the higher potency (entecavir, telbivudine, and clevudine) and the lower potency (lamivudine and adefovir) NA groups. The baseline characteristics of the patients are shown in Table 1. For both hepatitis B e antigen (HBeAg)-positive and HBeAg-negative patients, the gender ratio, age, alanine aminotransferase, qHBsAg, serum HBV DNA, intrahepatic total HBV DNA, cccDNA levels, and histology scores were comparable between the more potent and less potent NA groups. All patients had either HBV genotype B or C. HBV genotype distribution was comparable between the 2 groups, except that in HBeAg-positive patients, there was a higher proportion of HBV genotype C in the less potent NA group.

### *Correlation Between Baseline Quantitative Hepatitis B Surface Antigen, Serum Hepatitis B Virus DNA, and Intrahepatic Hepatitis B Virus DNA*

The correlations between baseline qHBsAg, serum HBV DNA, intrahepatic HBV DNA, and cccDNA levels are shown in Figure 1. In the HBeAg-positive patients, baseline qHBsAg correlated well with serum HBV DNA ($r = 0.562$, $P < .001$) and poorly with cccDNA ($r = 0.28$, $P = .022$) but did not correlate with intrahepatic total HBV DNA ($r = 0.078$, $P = .529$). In the HBeAg-negative patients, qHBsAg correlated poorly with serum HBV DNA ($r = 0.281$, $P = .034$) and did not correlate with intrahepatic total HBV DNA ($r = 0.003$, $P = .970$) or cccDNA ($r = 0.024$, $P = .860$). A stronger correlation between qHBsAg and other viral parameters was found in patients with HBV genotype B than in patients with genotype C, especially in the HBeAg-positive patients (Figure 1).