ĐIỀU TRỊ LIÊN TỤC ENTECAVIR CHO BỆNH NHÂN VGSV B MẠN TÍNH MỚI TRONG THỰC TẾ, KẾT QUẢ 6 NĂM

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GIỚI THIỆU:

Ức chế siêu vi mạnh và bền vững là một trong những mục tiêu điều trị của viêm gan siêu vi B mạn tính

Ức chế siêu vi hiệu quả có thể ngăn ngừa xơ gan và ung thư biểu mô tế bào gan, đó là mục tiêu dài hạn

Entecavir Ức chế siêu vi hiệu quả đến năm thứ 5

Có ít dữ liệu trong việc không dùng gián đoạn entecavir trong điều trị cho bệnh nhân VGSV B mạn tính mới hơn 5 năm

MỤC TIÊU:

xác định huyết thanh, các phản ứng sinh hóa, khả năng đáp ứng của virus và tỷ lệ đề kháng khi dùng liên tục entecavir (Baraclude) 6 năm

PHƯƠNG PHÁP:

222 bệnh nhân VGSV B mạn tính mới được điều trị bằng entecavir 0.5 mg mỗi ngày liên tục trong 6 năm (trong thực tế)

Xác định tỷ lệ cộng dồn sự chuyển đổi huyết thanh HBeAg, bình thường hóa men gan ALT, HBV DNA dưới ngưỡng phát hiện, bùng phát (tăng >1log HBV DNA từ mức thấp nhất) và rào cản di truyền kháng entecavir

Đặc điểm lâm sàng cơ bản của tất cả 222 bệnh nhân

Tuổi (khoảng), số tuổi	45 (21-77)
Nam	157 (70,7%)
HBeAg (+)	90 (40.5%)
HBV DNA (khoảng), log UI/ml	6.4 (3.32 ->8.1)
Albumin, umol/L	42 (22 – 50)
Bilirubin, umol/L	13 (2 – 216)
ALT (khoảng), U/L	92(17 – 2.168)
Số bệnh nhân với mức ALT cao	181 (81.5%)
Số bệnh nhân có dấu hiệu kháng lamivudine	2 (0.9%)
Số bệnh nhân có dấu hiệu kháng entecavir	0 (0%)

KẾT QUẢ:

- 99% bệnh nhân không còn phát hiện thấy HBV DNA sau 6 năm điều trị liên tục bằng entecavir
- 76% bệnh nhân có HBeAg (+) có sự chuyển đổi huyết thanh

- 95% bệnh nhân đạt ALT mức bình thường
- 2 bệnh nhân đạt khả năng sạch HBsAg
- Tỷ lệ bùng phát virus là 6.3%
- Tỷ lệ cộng dồn kháng thuốc sau 6 năm là 1.2%

KẾT LUẬN:

Entecavir là một thuốc ức chế siêu vi mạnh, bền vững và an toàn

Tỷ lệ cọng dồn kháng thuốc thấp, chỉ 1.2% sau sáu năm điều trị liên tục

Tỷ lệ làm sạch HbsAg thấp

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HEPATOLOGY

Changes of HBsAg and HBV DNA levels in Chinese chronic hepatitis B patients after 5 years of entecavir treatment

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Key words
entecavir, ETV, HBsAg, nucleoside analogue, surface antigen.

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The authors declare they have participated in the preparation of the manuscript and have seen and approved the final version.

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Abstract

Background and Aim: Hepatitis B surface antigen (HBsAg) kinetics during long-term entecavir therapy has not been well investigated.

Methods: We described the cumulative serologic, virologic, and biochemical outcomes and the occurrence of signature entecavir mutations among 222 Chinese treatment-naïve chronic hepatitis B (CHB) patients receiving entecavir for up to 5 years.

Results: The median rate of HBsAg reduction over 5 years was 0.125 log IU/mL/year. Patients with high baseline HBV DNA levels (≥ 8 log copies/mL or ≥ 7.3 log IU/mL), when compared with those with baseline hepatitis B virus (HBV) DNA < 7.3 log IU/mL, had a significantly greater median rate of HBsAg reduction (0.178 and 0.102 log IU/mL/year, respectively, $P < 0.001$). The difference in HBsAg decline was most prominent in the first year (0.324 and 0.062 log IU/mL/year, respectively, $P < 0.001$). Greater median rates of HBsAg reduction were also found in hepatitis B e antigen (HBeAg)-positive patients when compared with HBeAg-negative patients (0.144 and 0.098 log IU/mL/year, $P = 0.015$), and in patients with high baseline HBsAg levels (≥ 3 log IU/mL), when compared with patients with low baseline HBsAg < 3 log IU/mL (0.131 and 0.045 log IU/mL/year, respectively, $P = 0.001$). The 5-year cumulative rate of HBV DNA undetectability (< 20 IU/mL) was 97.1%. There were two cases of entecavir resistance, resulting in a 5-year cumulative resistance rate of 1.2%.

Conclusion: In contrast to the profound HBV DNA suppression, long-term entecavir treatment achieved only a slow decline in serum HBsAg. Although certain patient subgroups exhibit a more rapid HBsAg reduction, additional therapeutic agents are needed to increase the chance of HBsAg seroclearance in CHB.

Introduction

The management of chronic hepatitis B (CHB) has been revolutionized by the introduction of nucleoside analogue therapy. Long-term nucleoside analogue therapy has been proven to reduce liver-related complications and improve clinical outcomes.[1,2] Entecavir, a potent inhibitor of the hepatitis B virus (HBV), has been demonstrated to achieve successful long-term virologic suppression[3–5] and improve liver histology,[6] with only minimal problems of resistance in nucleoside-naïve patients.[7]

The quantification of serum hepatitis B surface antigen (HBsAg) is advocated as a marker of disease activity in CHB. Serum HBsAg measurements have been proposed to be used in identifying inactive carriers,[8] determining the risk of hepatocellular carcinoma,[9] correlating with histologic severity,[10] and predicting subsequent probability of HBsAg seroclearance.[11] A serum HBsAg level of < 1000 IU/mL (or < 3 log IU/mL) has been proposed to be a marker of good immune control in CHB.[8]

Although serum HBsAg levels could play a role in predicting favorable outcomes during pegylated interferon therapy,[12] its applicability in monitoring patients on nucleoside analogue therapy has not been well defined. Studies using entecavir or tenofovir with short duration of treatment (< 2 years) showed that in spite of potent and fast virologic suppression, serum HBsAg levels decline slowly.[13,14] A recent study demonstrated HBsAg < 3 log IU/mL to predict favorable outcomes among patients responding favorably to 10 years of lamivudine therapy.[15] Studies investigating the applicability of the above cut-off HBsAg level among CHB patients taking entecavir or tenofovir, as well as HBsAg

kinetics studies among such patients for longer durations, are still lacking.

We had previously reported a 3-year follow-up study on the virologic efficacy of continuous entecavir in treatment-naive Chinese CHB patients,[4] in which we found patients with high HBV DNA levels of > 8 log copies/mL (or ≥ 7.3 log IU/mL) took longer periods to achieve undetectable HBV DNA levels. The primary aim of our current extended study was to investigate the changes in serum HBsAg kinetics over 5 years of continuous entecavir therapy in the same patient cohort. The secondary aims were to examine the HBV DNA suppression, hepatitis B e antigen (HBeAg) seroconversion, normalization of alanine aminotransferase (ALT) levels, and the rate of drug resistance.

Methods

Patients. The current study recruited all treatment-naive CHB patients who were started on entecavir 0.5 mg daily in the Department of Medicine, Queen Mary Hospital, The University of Hong Kong from July 2005 to November 2007. All patients were HBsAg positive for at least 6 months before treatment. Patients were started on entecavir based on the following criteria: (i) HBeAg-positive non-cirrhotic patients with elevated ALT levels > upper limit of normal (ULN) and HBV DNA > 20 000 IU/mL; (ii) HBeAg-negative non-cirrhotic patients with elevated ALT > ULN and HBV DNA > 2000 IU/mL; and (iii) HBV DNA > 2000 IU/mL for patients with clinical evidence of cirrhosis. Patients with the following concomitant conditions were excluded: hepatitis C and D infection, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, and significant intake of alcohol (20 grams per day for female; 30 grams per day for male). Before April 2011, the prescription of entecavir in Hong Kong was not financially subsidized by the local health authorities; patients who opted to stop entecavir would be followed up closely with the date of stopping treatment deemed as the end of follow-up of this particular study. The study was approved by the Institutional Review Board of the University of Hong Kong and the Hospital Authority Hong Kong West Cluster, Hong Kong.

Patients were followed up every 3 to 6 months for clinical assessment and measurement of liver biochemistry and alpha-fetoprotein. Serum HBV DNA and HBsAg levels were performed at baseline and at every year after commencement of entecavir. Viral mutational analysis was performed for all samples from year 1 to 5 with detectable viremia (HBV DNA > 20 IU/mL). The occurrence of virologic breakthrough, defined as an increase of HBV DNA levels by at least 1 log IU/mL from the nadir for patients with detectable HBV DNA levels, or HBV DNA levels increasing to > 20 IU/mL for patients with undetectable HBV DNA levels, was recorded. Patients developing signature entecavir resistance were switched to tenofovir monotherapy, with the date of therapy change regarded as the date of last follow-up.

Laboratory assays. Serum HBeAg, antibody to HBeAg (anti-HBe) and antibody to HBsAg (anti-HBs) were measured by Abbott Laboratories (Chicago, IL, USA). Serum HBsAg levels were performed using Elecsys HBsAg II assay (Roche Diagnostics, Gmbh, Mannheim), with a linear range of 0.05 to 52 000 IU/mL. Samples with levels higher than 52 000 IU/mL were retested at a dilution of 1:100 according to the manufacturer's instruction. Serum HBV DNA levels were measured using Cobas Taqman assay (Roche Diagnostics, Branchburg, NJ, USA) with the lower limit of detection of 20 IU/mL.

Resistance profile was performed using a line probe assay (LiPA, Innogenetics NV, Gent, Belgium), with both LiPA DR versions 2 and 3 used to identify the amino acids at codons rt173, rt180, rt204, and rt184, rt202, and rt250, respectively. Genotypic resistance to entecavir was defined by the presence of three viral mutations: rtL180M, rtM204V/I, and one of the following: rtT184S/C/GA, rtS202G/C/I, or rtM250V.

Statistical analyses. Continuous variables were expressed as median (range). Serum HBsAg and HBV DNA levels were expressed in logarithm. The comparison of continuous variables was performed using the Mann–Whitney U test. Categorical variables were compared using Pearson's chi-squared test or Fisher's exact test as appropriate. The cumulative rates of virologic suppression (HBV DNA < 20 IU/mL), HBeAg seroconversion, ALT normalization, and virologic breakthrough were calculated using the Kaplan–Meier method; differences were determined using the log-rank test. The cumulative rate of development of viral resistance was calculated from the formula: $P = 1\text{-}(1\text{-}n1/N1)(1\text{-}n2/N2)\ldots(1\text{-}nx/Nx)$.[16] Correlation between different clinical parameters was performed using Spearman's bivariate correlation. All statistical analyses were performed using SPSS version 19.0 (SPSS Inc, Chicago, IL, USA). A two-sided P value of < 0.05 was considered statistically significant.

Results

Two hundred and twenty-two treatment-naive CHB patients were recruited, with their baseline demographics illustrated in Table 1. Ninety (40.5%) and 181 (81.5%) patients were HBeAg positive and had elevated baseline ALT, respectively. There were no signature entecavir mutations identified at baseline. Seventy-one patients (32.0%) had high HBV DNA levels (defined as ≥ 8 log

Table 1 Baseline parameters of all 222 patients

Age (range), years	47 (21–77)
Number of male	157 (70.7%)
Number of HBeAg-positive patients	90 (40.5%)
HBV DNA (range), log IU/mL	6.40 (3.32–> 8.10)
HBV DNA ≥ 7.3 log IU/mL	71 (32.0%)
HBsAg (range), log IU/mL	3.41 (0.96–5.88)
Number of patients with HBsAg ≥ 3 log IU/mL	173 (77.9%)
Albumin, g/L	42 (22–50)
Bilirubin, umol/L	13 (2–216)
ALT (range), U/L	92 (17–2168)
Number of patients with elevated ALT level	181 (81.5%)
Number of patients with lamivudine signature mutations	2 (0.9%)
Number of patients with entecavir signature mutations	0 (0%)

Continuous variables expressed as median (range).
ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV DNA, hepatitis B virus DNA.

copies/mL or ≥ 7.3 log IU/mL) at baseline, of which 48 (67.6%) were HBeAg positive, significantly more than patients with baseline HBV DNA < 7.3 log IU/mL (27.8%, $P < 0.001$). All 222 patients were followed up to year 1; 188, 173, 170, and 156 patients were followed up to years 2, 3, 4, and 5, respectively.

Baseline HBsAg levels correlated well with baseline HBV DNA levels in all patients (r = 0.572, $P < 0.001$) and HBeAg-positive patients (r = 0.689, $P < 0.001$). Moderate correlation between baseline HBsAg and HBV DNA levels also existed in HBeAg-negative patients (r = 0.375, $P < 0.001$).

HBsAg kinetics. The median rate of HBsAg reduction among patients with 5 years of follow-up ($n = 156$) was 0.125 (range—0.261–0.590) log IU/mL/year. When comparing the median rates of HBsAg reduction of every individual year, there was a significant difference between years 1 and 5 (0.128 and 0.092 log IU/mL/year, respectively, $P = 0.027$), but not when comparing year 1 with year 2 (0.081 log IU/mL/year, $P = 0.128$), year 3 (0.095 log IU/mL/year, $P = 0.072$), or year 4 (0.151 log IU/mL/year, $P = 0.948$).

The median rates of HBsAg reduction among patients with high-baseline HBV DNA levels (≥ 7.3 log IU/mL or ≥ 8 log copies/mL) ($n = 54$) and patients with baseline HBV DNA levels < 7.3 log IU/mL ($n = 102$) among patients with 5 years of follow-up is depicted in Figure 1a. Patients with high baseline HBV DNA levels, when compared with the patients with intermediate to low baseline HBV DNA levels, had a significantly greater rate of HBsAg reduction (0.178 and 0.102 log IU/mL/year, respectively, $P < 0.001$) (Fig. 1a). The difference in the rate of HBsAg reduction was most striking at year 1 (0.324 and 0.062 log IU/mL/year, respectively, $P < 0.001$). When comparing the median rates of HBsAg reduction among these two groups of patients from the second to fifth year, there was no significant difference (0.099 and 0.100 log IU/mL/year, respectively, $P = 0.985$).

The median rate of HBsAg reduction over 5 years among patients stratified by HBeAg status is depicted in Figure 1b. HBeAg-positive patients ($n = 61$), when compared with HBeAg-negative patients ($n = 95$), had a greater rate of HBsAg decline (0.144 and 0.098 log IU/mL/year, respectively, $P = 0.015$). The difference in the HBsAg reduction rate in the first year had a trend toward significance (0.209 and 0.089 log IU/mL/year, respectively, $P = 0.071$). From the second to fifth year, there was no significant difference in the rate of HBsAg reduction (0.111 and 0.099 log IU/mL/year, respectively, $P = 0.365$). Patients who achieved HBeAg-seroconversion ($n = 40$), when compared with persistently HBeAg-positive patients ($n = 21$), had a significantly lower median HBsAg level at baseline (3.82 and 4.67 log IU/mL, respectively, $P = 0.009$) and year 1 (3.54 and 3.86 log IU/mL, respectively, $P = 0.015$). There was no significant difference in the rate of HBsAg decline when comparing persistently HBeAg-positive patients with patients achieving HBeAg seroconversion (0.191 and 0.136 log IU/mL/year, respectively, $P = 0.198$).

We also compared the median rate of HBsAg reduction over 5 years among patients stratified by baseline HBsAg levels (Fig. 1c). Patients with baseline HBsAg ≥ 3 log IU/mL ($n = 129$), when compared with patients with baseline HBsAg < 3 log IU/mL ($n = 27$), had a significantly greater rate of HBsAg decline (0.131 and 0.045 log IU/mL/year, respectively, $P = 0.001$).

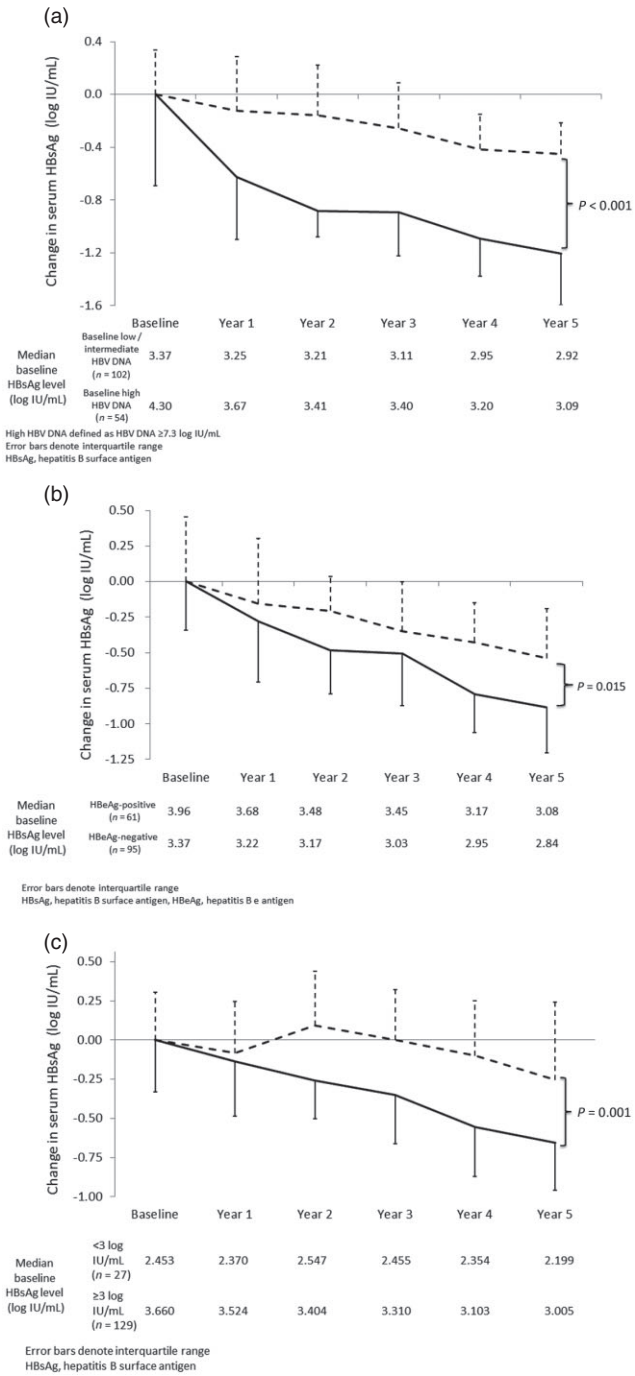

Figure 1 Serum median hepatitis B surface antigen (HBsAg) reduction from baseline as stratified by (a) baseline hepatitis B virus (HBV) DNA levels. – –, Baseline low/intermediate HBV DNA; ——, Baseline high HBV DNA, (b) hepatitis B e antigen (HBeAg) status. ——, HBeAg-positive; – –, HBeAg-negative, and (c) baseline HBsAg levels among patients followed up for 5 years. – –, Baseline HBsAg < 3 log IU/mL; ——, Baseline HBsAg ≥ 3 log IU/mL.

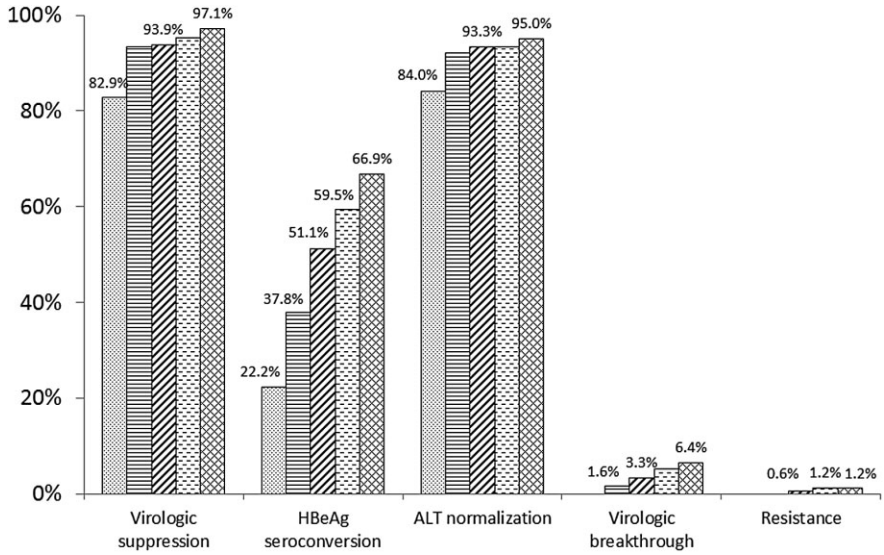

Figure 2 Cumulative rates of virologic, serologic, and biochemical outcomes and rate of resistance up to year 5. ▨, Year 1; ▤, Year 2; ▨, Year 3; ▦, Year 4; ▩, Year 5. Cumulative rates estimated by the Kaplan–Meier method. Rate of resistance calculated as suggested by Pawlotsky *et al*. Virologic suppression defined as HBV DNA < 20 IU mL. ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen.

Only 1 patient (35 years old male, baseline HBeAg negative) achieved HBsAg seroclearance. His baseline HBsAg was 1.81 log IU/mL, with HBsAg clearance at year 2, and detectable anti-HBs (370 mIU/mL) at year 3. No other patients achieved HBsAg seroclearance during the follow-up period.

Virologic suppression. The cumulative rates of HBV DNA becoming undetectable (HBV DNA < 20 IU/mL) from year 1 to year 5 is depicted in Figure 2. There was an incremental increase in the rates of HBV DNA becoming undetectable from 82.9% in year 1 to 97.1% in year 5.

When stratifying patients based on their baseline HBV DNA levels, patients with high baseline HBV DNA levels, that is, HBV DNA ≥ 7.3 log IU/mL, when compared with patients with HBV DNA levels < 7.3 log IU/mL, had a significantly lower cumulative rate of HBV DNA becoming undetectable up to 5 years (Fig. 3, $P = 0.006$). The difference between the two groups of patients was most marked at years 1 (70.4% and 88.7%, respectively) and 2 (86.0% and 96.8%) while there was little difference in the rates of HBV DNA becoming undetectable up to year 5 (97.3% and 100%, respectively).

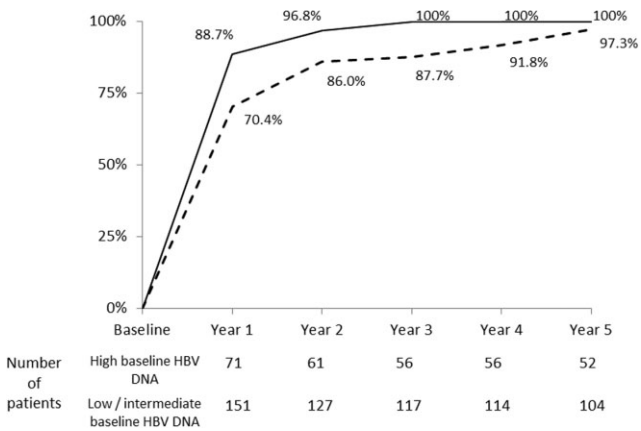

Figure 3 Cumulative rates of virologic suppression up to year 5 stratified by baseline hepatitis B virus (HBV) DNA levels. ▬ ▬, Baseline high HBV DNA; ——, Baseline low/intermediate HBV DNA. Cumulative rates estimated by the Kaplan–Meier method ($P = 0.006$). High HBV DNA defined as HBV DNA ≥ 7.3 log IU/mL. Virologic suppression defined as HBV DNA < 20 IU mL.

HBeAg seroconversion and ALT normalization. The 5-year cumulative rate of HBeAg seroconversion among HBeAg-positive patients ($n = 90$) and the 5-year cumulative rate of ALT normalization among patients with elevated ALT ($n = 181$) were 66.9% and 95.0%, respectively (Fig. 2).

Virologic breakthrough and resistance profile. The cumulative rate of virologic breakthrough up to year 5 was 6.4% (Fig. 2). Altogether, five patients had virologic breakthroughs of which two patients developed genotypic resistance to entecavir at years 3 and 4, respectively (Table 2). Serum ALT remained normal for both patients. Both patients were switched to tenofovir with HBV DNA becoming undetectable documented 2 to 3 months after changing of therapy. The remaining three patients were found to be noncompliant to entecavir therapy. The cumulative rate of resistance to entecavir up to year 5 was 1.2%.

Safety and clinical complications. Eight patients (3.6%) developed HCC after a median duration of 21 months (range 13 to 43 months), of which six patients were cirrhotic at baseline. Among these eight patients, five remained in remission after partial hepatectomy, two required liver transplantation, and one subsequently died after 22 months of entecavir. There was one patient who died of sub-arachnoid hemorrhage after 38 months of follow-up; there were otherwise no cases of liver-related mortality noted.

Forty-nine patients discontinued entecavir therapy, with the reasons being unwilling to commit to long-term treatment

Table 2 Clinical profiles of the two patients developing genotypic resistance to entecavir

Patient number	Sex/Age	HBeAg status		HBV DNA levels (log IU/mL)/ALT levels (U/L)					Entecavir-resistant mutations
		Baseline	Last follow-up	Baseline	Year 1	Year 2	Year 3	Year 4	
1	M/45	Positive	Negative	> 8.11/825	1.6/39	UD/36	2.51/34	–	rt180M rt204V rt184 S/C/G/A
2	M/52	Positive	Positive	> 8.11/57	3.92/42	2.88/44	2.75/33	3.21/17	rt180M rt204V rt184 I/F/M

Genotypic resistance to entecavir was detected for patients 1 and 2 at years 3 and 4, respectively.
HBeAg, hepatitis B e antigen; UD, undetectable.

($n = 26$), financial difficulties ($n = 20$), development of signature entecavir mutations ($n = 2$), and switching to telbivudine because of pregnancy ($n = 1$). No patients stopped entecavir due to side effects.

Discussion

This present study reports the 5-year efficacy of entecavir in treatment-naïve CHB patients in a real-world scenario, where practical issues including compliance of therapy and cost could influence therapeutic efficacy.[17] Besides, our study demonstrated several features of HBsAg kinetics during entecavir therapy which could be relevant to the probability of HBsAg seroclearance, the ultimate treatment endpoint in CHB.

The median rate of HBsAg reduction in this study was 0.125 log IU/mL/year, which was similar to results from natural history studies[11] and studies involving other nucleoside analogues.[15] As nucleoside analogue therapy has only a minimal effect on the transcription of HBsAg from covalently closed circular DNA (cccDNA) and on the secretion of empty HBsAg particles,[18,19] serum HBsAg declines at a slow rate despite rapid and potent virologic suppression. Therefore, nucleoside analogue therapy should be given on a long-term basis when targeting the goal of HBsAg seroclearance. In addition, a preliminary study reported that even in patients who achieved undetectable HBV DNA levels, 78.1% of patients would have HBV DNA rebound (defined as > 2000 IU/mL) after stopping of entecavir, with HBsAg levels failing to predict off-treatment virologic remission after treatment cessation.[20] These phenomena suggest that in spite of the positive correlation between HBsAg and HBV DNA levels at baseline as demonstrated in the present and other studies,[21,22] the viral activities of these two markers respond differently toward antiviral therapy. Therefore, these two viral surrogate markers cannot be used in this setting to reflect or replace one and the other. In the future, treatment for CHB may be ideal to have a potent nucleoside analogue to achieve virologic suppression plus an agent that can profoundly inhibit HBsAg production.[23]

Nonetheless, specific patient groups do have a greater rate of HBsAg decline. From our study results, patients with baseline high HBV DNA levels (≥ 7.3 log IU/mL) had a significantly greater rate of HBsAg reduction (Fig. 1a), with a markedly greater rate of decline during the first year of therapy (0.324 log IU/mL/year), although the reduction rate in subsequent years dropped back to approximately 0.1 log IU/mL/year, similar to the rest of the patient cohort. HBeAg-positive patients, when compared with HBeAg-negative patients, also had a more significant reduction of HBsAg level (Fig. 1b) similar to the phenomenon observed in patients with high and low baseline HBV DNA levels. This clinical phenomenon can be explained by the nature of HBsAg production.[18] After going through the process of transcription from cccDNA and subsequent translation, HBsAg particles are released into the peripheral blood stream either in the form of episomal HBV DNA-containing virions or as empty HBsAg subviral particles. When patients with high HBV DNA levels (which include a large number of HBeAg-positive patients) were started on entecavir, potent suppression of viral replication would lead to a rapid decline of episomal HBV DNA-containing virions, resulting in an increased rate of HBsAg reduction in the first year of therapy. However, nucleoside analogue therapy would have little effect on the remaining empty subviral HBsAg particles in the circulation, explaining why the rate of HBsAg reduction is markedly diminished during subsequent therapy.

Another important finding is the reduced HBsAg decline rate among patients with baseline HBsAg < 3 log IU/mL (Fig. 1c, 0.045 log IU/mL/year). During nucleoside analogue therapy, the proportion of cccDNA as total intrahepatic HBV DNA would increase because cccDNA is amplified by the transport of episomal HBV DNA back into the nucleus,[24] resulting in slower rates of serum HBsAg decline. This could partially explain the difficulty in achieving HBsAg seroclearance during nucleoside analogue therapy.

In our real-world scenario, continuous entecavir over 5 years achieved very high rate of virologic suppression and low incidence of resistance. Although patients with high baseline HBV DNA levels (HBV DNA ≥ 7.3 log IU/mL) had only a cumulative virologic suppression rate of 70.4% at year 1, this has increased to over 90% by year 4. The significance of a more rapid virologic suppression among patients with high baseline HBV DNA is still up to debate. While rapid virologic suppression may be important when nucleoside analogues with a low barrier to resistance are used,[25,26] and remains essential in patients with acute-on-chronic liver failure,[27] its impact is minimal when using potent antiviral agents such as entecavir and tenofovir, especially because the majority of patients with on-treatment detectable viremia would still achieve virologic suppression in the long run.[28] This is also supported by a recent study demonstrating the antiviral efficacy of entecavir and tenofovir combination to be non-superior to entecavir monotherapy up to 96 weeks.[29] Another study also demonstrates that 240 weeks of tenofovir is equally effective in suppression of HBV DNA in patients with high baseline viral load of ≥ 9 $\log_{10}$

copies/mL and those with lower baseline viral load of < 9 $\log_{10}$ copies/mL (98.3% and 99.2% with HBV DNA < 400 copies/mL, respectively).[30]

A limitation of our current study was the lack of HBV genotyping data. Nevertheless, among Chinese CHB patients in whom the majority of patients are of genotype B or C, HBV genotyping is not an important determinant of HBsAg kinetics.[11,15,31] In addition, our current patient cohort had only one patient achieving HBsAg seroclearance. Hence, to analyze the predictive value of HBsAg kinetics for subsequent HBsAg seroclearance, studies of even longer duration would be needed. Future studies could also explore the correlation of intrahepatic cccDNA with serum HBsAg decline, especially among those achieving subsequent HBsAg seroclearance.

In conclusion, serum reduction of HBsAg levels was slow and gradual in spite of the profound HBV DNA reduction achieved in patients receiving 5 years of entecavir therapy. Achieving the goal of HBsAg seroclearance requires long durations when nucleoside analogues are used as the sole agents for the treatment of CHB. The development of new agents capable of achieving strong HBsAg reduction would be necessary to enhance the current armamentarium of HBV treatment. Combination of these two different groups of agents may be the future ideal therapy for CHB.

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