Antiviral activity, dose–response relationship, and safety of entecavir following 24-week oral dosing in nucleoside-naïve Japanese adult patients with chronic hepatitis B: a randomized, double-blind, phase II clinical trial

Michiko Shindo · Kazuaki Chayama · Satoshi Mochida · Joji Toyota · Eiichi Tomita · Hiromitsu Kumada · Osamu Yokosuka · Michio Sata · Norio Hayashi · Kazuyuki Suzuki · Takeshi Okanoue · Hirohito Tsubouchi · Hiroki Ishikawa · Taku Seriu · Masao Omata

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Abstract

Purpose A randomized, double-blind, multicenter study (ETV-047) was conducted to evaluate the dose–response relationship of entecavir and compare its antiviral activity and safety with lamivudine in Japanese patients with chronic hepatitis B (CHB).

Methods One hundred thirty-seven nucleoside-naïve adult patients with CHB were randomized to once-daily

oral doses of entecavir 0.01, 0.1, or 0.5 mg or lamivudine 100 mg for 24 weeks. The primary efficacy end point used to evaluate the dose–response relationship was mean change from baseline in serum hepatitis B virus (HBV) DNA level at week 22, as determined by polymerase chain reaction assay.

Results Entecavir demonstrated a clear dose–response relationship, with mean change from baseline in serum

M. Shindo (✉)
Division of Liver Disease, Department of Internal Medicine,
Akashi Municipal Hospital, 1-33 Takasyo-machi, Akashi-shi,
Hyogo, Japan
e-mail: mshindo@skyblue.ocn.ne.jp

K. Chayama
Department of Medicine and Molecular Science, Graduate
School of Biomedical Sciences, Hiroshima University,
Hiroshima, Japan

S. Mochida
Department of Gastroenterology and Hepatology, Saitama
Medical University, Saitama, Japan

J. Toyota
Department of Hepatology, Sapporo Kosei General Hospital,
Hokkaido, Japan

E. Tomita
Department of Gastroenterology, Gifu Municipal Hospital, Gifu,
Japan

H. Kumada
Department of Hepatology, Toranomon Hospital, Tokyo, Japan

O. Yokosuka
Department of Medicine and Clinical Oncology, Graduate
School of Medicine, Chiba University, Chiba, Japan

M. Sata
Department of Gastroenterology, Kurume University School of
Medicine, Fukuoka, Japan

N. Hayashi
Department of Gastroenterology and Hepatology, Osaka
University Graduate School of Medicine, Osaka, Japan

K. Suzuki
Department of Internal Medicine, Iwate Medical University,
Iwate, Japan

T. Okanoue
Department of Gastroenterology, Saiseikai Suita Hospital,
Osaka, Japan

H. Tsubouchi
Department of Digestive Disease and Lifestyle-Related Disease,
Kagoshima University Graduate School of Medical and Dental
Sciences, Kagoshima, Japan

H. Ishikawa · T. Seriu
Pharmaceutical Research Institute, Bristol-Myers Squibb Japan,
Tokyo, Japan

M. Omata
Department of Gastroenterology, Graduate School of Medicine,
University of Tokyo, Tokyo, Japan

HBV DNA level of -3.11 , -4.77 , and -5.16 $\log_{10}$ copies/ml with entecavir 0.01, 0.1, and 0.5 mg, respectively. Entecavir 0.5 mg was superior to lamivudine 100 mg for the mean change in HBV DNA level (-5.16 vs. -4.29 $\log_{10}$ copies/ml; $P = 0.007$). The overall incidence of adverse events was comparable between treatment groups. Two patients discontinued treatment because of adverse events (one with liver cirrhosis [entecavir 0.5 mg] and one with grade 4 serum alanine aminotransferase (ALT) elevation, nausea, and malaise [lamivudine 100 mg]). Serum ALT flares were observed in four patients; flares were associated with 2 $\log_{10}$ reductions or more in HBV DNA level and resolved without dose interruption.

Conclusion Entecavir 0.01–0.5 mg is well tolerated and produces a dose-dependent reduction in viral load in nucleoside-naïve Japanese patients with CHB. Compared with lamivudine 100 mg, entecavir 0.1 mg demonstrated noninferiority and entecavir 0.5 mg was superior in this population.

Keywords Chronic hepatitis B · Entecavir · Lamivudine · HBV DNA · ALT flare

Introduction

It is reported that more than 2 billion individuals worldwide have been infected with hepatitis B virus (HBV) and approximately 350 million people are long-term HBV carriers [1]. Chronic hepatitis B (CHB) is induced by chronic replication of HBV in the liver and has a poor prognosis, with 20–40% of infected individuals developing liver cirrhosis, noncompensated liver disorder, or hepatocellular carcinoma [2]. Treatment of CHB is aimed at sustained inhibition of HBV replication and remission of liver disease [3], ultimately preventing progression to liver cirrhosis or hepatocellular carcinoma [4].

Prior to the advent of the nucleoside analog lamivudine, interferon- α formed the mainstay of treatment, but this immunoregulatory cytokine requires parenteral administration and is poorly tolerated [5]. Lamivudine is well tolerated on oral administration and has been proven to be highly effective in the treatment of CHB, but the emergence of resistance mutations (including the YMDD motif) in the reverse-transcriptase domain of HBV polymerase frequently results in overt viral rebound and disease progression [6–9]. The novel nucleoside analog adefovir is effective against wild-type HBV and lamivudine-resistant strains and is well tolerated on long-term administration, but its clinical use is restricted by the need for renal monitoring in patients with impaired renal function [10].

Entecavir, a cyclopentylguanine-derived nucleoside analog and selective inhibitor of HBV replication, was

approved by the U.S. Food and Drug Administration in 2005 for the treatment of CHB. Entecavir displays potent antiviral activity in the woodchuck and duck models of HBV infection [11, 12] and is reported to be 100- to 2,200-fold more potent than lamivudine and adefovir in inhibiting HBV replication in vitro [13, 14]. Phase II clinical trials of entecavir conducted in non-Japanese patients with CHB have demonstrated entecavir to be well tolerated and more effective than lamivudine [15, 16].

A global dose-finding study (ETV-005) conducted in lamivudine-naïve patients with CHB compared three doses of entecavir (0.01, 0.1, and 0.5 mg once daily) with lamivudine 100 mg once daily over a 22-week treatment period. Entecavir showed a clear dose–response relationship and was well tolerated at all three dose levels; in addition, 0.1 and 0.5 mg of entecavir showed superior antiviral activity compared with 100 mg of lamivudine [15].

Phase I studies of single-dose (0.05–2.5 mg) and multiple-dose (0.1–1.0 mg daily) entecavir conducted in Japan have confirmed the drug's safety in healthy men. As in Caucasian populations, entecavir displayed linear plasma pharmacokinetics over a wide range of doses, including putative therapeutic doses (0.5 and 1.0 mg), in Japanese subjects; there was no evidence of significant ethnic differences in its pharmacokinetics and pharmacodynamics. Similar findings to those obtained in the global phase II clinical trials of entecavir might therefore be expected from corresponding studies conducted in Japanese patients.

To evaluate the dose–response relationship, the antiviral activity and safety of entecavir in Japanese CHB patients, we conducted a 24-week phase II study comparing entecavir (0.01, 0.1, and 0.5 mg daily) to lamivudine (100 mg daily).

Materials and methods

Study design

This randomized, double-blind, double-dummy study was conducted at 38 institutions in Japan from August 2003 to March 2005. Eligible patients comprised 20- to 75-year-old men and women with CHB who fulfilled the following criteria: (i) HBsAg-positive for 24 weeks or more or IgM HBcAb-negative with biopsy-confirmed CHB; (ii) HBeAg-positive or HBeAg-negative for 12 weeks or more; (iii) serum HBV DNA level 40 MEq/ml or more (143 pg/ml) by QuantiplexTM branched DNA hybridization method (bDNA assay) (≥ 7.6 $\log_{10}$ genome equivalent by the transcription-mediated amplification method or $\geq 10^{7.6}$ copies/ml by Roche AmplicorTM polymerase chain reaction method [PCR assay]) measured 2 weeks or more before screening and serum HBV DNA level 40 MEq/ml or more (by bDNA assay) at screening; (iv) serum alanine

aminotransferase (ALT) level 1.25–10 times the upper limit of normal (ULN); and (v) well-compensated liver disease with prothrombin time prolongation 3 s or less or international normalized ratio 1.5 or less, serum albumin level 3.0 g/dl or more, and total bilirubin 2.5 mg/dl or less (42.75 μ mol/l). After a 6-week screening period, eligible patients were stratified according to HBeAg status and study site and randomized (1:1:1:1) to oral treatment with entecavir (0.01, 0.1, or 0.5 mg plus matching placebo capsule) or lamivudine (100 mg plus matching placebo tablet) once daily for 24 weeks. All doses were administered at fixed times of the day, avoiding the 2 h before and after meals. Pregnant women were excluded from the study, as were patients with liver cirrhosis, patients with a history or evidence of variceal bleeding, patients with hepatic encephalopathy or ascites requiring diuretics, or patients with paracentesis. Patients with other liver disease (e.g., autoimmune hepatitis) were excluded from the study. In addition, patients were excluded if they had a serum creatinine level more than $1.5 \times$ ULN, hemoglobin level less than 10.0 g/dl, platelet count less than 70,000/mm³, granulocyte count less than $<1,500/\text{mm}^3$ or plasma α -fetoprotein level more than 100 ng/ml, a history of allergy induced by nucleoside analog or exposure to nucleoside analogs, a recent history (previous 24 weeks) of treatment with immunosuppressives or interferon- α/β , or current treatment of CHB.

Treatment efficacy was assessed after 22 weeks, and all eligible patients who completed 24 weeks of blinded therapy were given the option of enrolling in a separate entecavir trial. Patients who discontinued therapy prematurely were followed up for 24 weeks postdosing. Patients began anti-HBV therapy as recommended by their physician during the postdosing follow-up period.

Informed consent was obtained from all patients in writing prior to their inclusion in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and notifications were issued by the Ministry of Health and Labor.

Efficacy and safety assessment

The primary efficacy end point for the evaluation of the dose–response relationship of entecavir was the change from baseline in mean serum HBV DNA level at week 22, as determined by PCR assay. Secondary efficacy end points for the assessment of the noninferiority of entecavir at each dose to lamivudine included the change from baseline in mean serum HBV DNA level at week 22, as determined by PCR assay, the percentage of patients with a reduction in serum HBV DNA level $2 \log_{10}$ copies/ml or more or a serum HBV DNA level below the limit of detection

(400 copies/ml by PCR assay; 2.5 pg/ml or 0.7 MEq/ml by bDNA assay) at week 22, the percentage of patients with HBeAg loss, the percentage of patients with HBeAg seroconversion (HBeAg loss and appearance of HBe-antibody), the percentage of patients achieving ALT normalization (World Health Organization grade 0: $<1.25 \times$ ULN), and the percentage of patients achieving a protocol-defined response (HBV DNA level <0.7 MEq/ml by bDNA assay, HBeAg negativity and serum ALT level $<1.25 \times$ ULN for HBeAg-positive patients; HBV DNA level <0.7 MEq/ml by bDNA assay and serum ALT level <1.25 ULN for HBeAg-negative patients) at week 22. The incidence of genotypic drug resistance was also assessed in patients who had a $1 \log_{10}$ copies/ml or more increase in HBV DNA by PCR from nadir while on study drug.

Based on the results of the global dose–response study of entecavir conducted in nucleoside-naïve patients (ETV-005 study) [15], noninferiority of entecavir 0.1 or 0.5 mg compared with lamivudine (100 mg) was confirmed if the upper 95% confidence interval (CI) for the difference in mean HBV DNA levels at week 22 was $0.8 \log_{10}$ copies/ml or less.

Assay methods

Serum HBV DNA level was determined by Roche AmplicorTM PCR assay (Roche Diagnostics K.K., Tokyo, Japan) and QuantiplexTM (Chiron) bDNA assay. Clinical laboratory tests, serum HBV DNA assays, and HBV serology were performed at the central clinical laboratory designated by the trial sponsor. Genotypic analysis of HBV isolates was performed using samples collected from patients on the first day of treatment. Genotypic analysis of HBV DNA polymerase was performed at SRL Inc. (Tokyo, Japan).

Statistical analysis

Numerical data were expressed by descriptive statistics. Serum HBV DNA level, a continuous variable, was analyzed after logarithmic transformation. For treatment group, comparisons of continuous variables, analysis of variance models, incorporating baseline HBV DNA level and HBeAg status as covariates were employed. For intertreatment comparisons of binary data, Cochran–Mantel–Haenszel tests were employed using baseline HBeAg status as a stratification factor. For analysis of dose–response relationships, Student's *t* test was applied to linear regression plots of serum HBV DNA level against log dose. A two-sided $P < 0.05$ was taken to indicate statistical significance. For analysis of dose–response relationships using efficacy data, a two-sided $P < 0.05/3$ was taken to

indicate statistical significance following Bonferroni adjustment.

Results

Study population and demographic characteristics

A total of 137 patients, including 20- to 73-year-old men and women, met the study eligibility criteria and were randomized to the following treatment groups: entecavir 0.01 mg ($n = 35$), entecavir 0.1 mg ($n = 34$), entecavir 0.5 mg ($n = 34$), and lamivudine 100 mg ($n = 34$). Three patients (two in the entecavir 0.5 mg group and one in the lamivudine 100 mg group) discontinued the study prematurely; the reasons for discontinuation were noncompliance (one patient in the entecavir 0.5 mg group) and adverse events (liver cirrhosis in one patient [entecavir 0.5 mg group] and grade 4 serum ALT elevation with nausea and malaise in one patient [lamivudine 100 mg group]). Accordingly, a total of 134 patients (entecavir 0.01 mg group, 35 patients; entecavir 0.1 mg group, 34 patients; entecavir 0.5 mg group, 32 patients; and lamivudine 100 mg group, 33 patients) completed 24 weeks of treatment and were included in the efficacy assessment.

The four treatment groups were matched with respect to gender, age, body weight, and proportion of HBeAg-positive patients (Table 1). Serum HBV DNA levels by PCR assay (mean $\pm$ SD) at baseline were 7.94 ± 0.87 , 8.09 ± 1.05 , 8.39 ± 0.73 , and 7.94 ± 0.83 log₁₀ copies/

ml for the entecavir 0.01, 0.1, and 0.5 mg and lamivudine 100 mg groups, respectively. With regard to HBV genotype, 124 patients were genotype C, 6 patients were genotype A, 5 patients were genotype B, and 2 patients were genotype F. All patients were nucleos(t)ide-naïve and none had been pretreated with interferon therapy.

Virologic response

Mean changes (from baseline) in serum HBV DNA level at week 22 were -3.11 , -4.77 , and -5.16 log₁₀ copies/ml with entecavir 0.01, 0.1, and 0.5 mg, respectively (Fig 1; Table 2). Estimated differences in serum HBV DNA levels between the 0.1 and 0.5 mg entecavir groups and the low-dose entecavir group (0.01 mg) were determined after adjustment for baseline level and HBeAg status. Estimated intertreatment group differences (adjusted 95% CI) were -1.61 (-2.20 to -1.02) log₁₀ copies/ml between the entecavir 0.01 and 0.1 mg groups and -1.95 (-2.53 to -1.37) log₁₀ copies/ml between the entecavir 0.5 and 0.01 mg groups; both of these differences were statistically significant ($P < 0.0001$). In contrast, the difference in serum HBV DNA levels between the high-dose (0.5 mg) and medium-dose (0.1 mg) entecavir groups was not statistically significant (estimated difference [adjusted 95% CI] -0.23 [-0.69 to 0.23] log₁₀ copies/ml). Taken together, these results demonstrate the superiority of high- and medium-dose entecavir (0.1 and 0.5 mg) compared with low-dose entecavir (0.01 mg) in terms of viral load reduction (Table 3). Linear regression analyses indicated a

Table 1 Baseline demographics and clinical characteristics of treated subjects

	ETV 0.01 mg ($n = 35$)	ETV 0.1 mg ($n = 34$)	ETV 0.5 mg ($n = 34$)	LVD 100 mg ($n = 34$)
Male, n (%)	25 (71.4)	23 (67.6)	23 (67.6)	28 (82.4)
Female, n (%)	10 (28.6)	11 (32.4)	11 (32.4)	6 (17.6)
Age (years), mean $\pm$ SD	42.0 $\pm$ 12.5	40.1 $\pm$ 9.8	39.8 $\pm$ 10.4	42.3 $\pm$ 12.6
Weight (kg), mean $\pm$ SD	66.2 $\pm$ 12.5	64.6 $\pm$ 11.9	65.3 $\pm$ 11.1	64.4 $\pm$ 9.0
Ethnicity Japanese, n (%)	35 (100)	34 (100)	34 (100)	34 (100)
HBV DNA (log ₁₀ copies/ml by PCR), mean $\pm$ SD	7.94 $\pm$ 0.87	8.09 $\pm$ 1.05	8.39 $\pm$ 0.73	7.94 $\pm$ 0.83
HBeAg positive, n (%)	30 (85.7)	30 (88.2)	30 (88.2)	31 (91.2)
ALT (IU/l), mean $\pm$ SD	150.1 $\pm$ 111.8	162.0 $\pm$ 127.1	142.4 $\pm$ 82.2	185.0 $\pm$ 130.8
AST (IU/l), mean $\pm$ SD	83.2 $\pm$ 40.0	114.3 $\pm$ 109.4	81.0 $\pm$ 43.0	121.6 $\pm$ 85.4
Total bilirubin (mg/dl), mean $\pm$ SD	0.65 $\pm$ 0.25	0.56 $\pm$ 0.15	0.66 $\pm$ 0.25	0.71 $\pm$ 0.28
HBV genotype (%)				
C	32 (91.4)	30 (88.2)	32 (94.1)	30 (88.2)
A	1 (2.86)	2 (5.88)	1 (2.94)	2 (5.88)
B	1 (2.86)	1 (2.94)	1 (2.94)	2 (5.88)
F	1 (2.86)	1 (2.94)	0	0

ETV entecavir; LVD lamivudine

Fig. 1 Mean change from baseline in serum HBV DNA level by PCR assay through 22 weeks in patients treated with entecavir (ETV) 0.01, 0.1, and 0.5 mg and lamivudine 100 mg. Mean change in serum HBV DNA level was plotted as a function of time after the initiation of the protocol therapy (weeks). Data expressed as mean $\pm$ SE

significant dose–response relationship between log₁₀ entecavir dose and reduction in log₁₀ serum HBV DNA level ($P < 0.0001$).

Mean change (from baseline) in serum HBV DNA level at week 22 for the lamivudine 100 mg group was -4.29 log₁₀ copies/ml (Fig. 1; Table 2). Estimated mean differences (95% CI) in serum HBV DNA level (after adjustment for baseline level and HBeAg status) were -0.39 (-0.83 to 0.05) log₁₀ copies/ml between the entecavir 0.1 mg and lamivudine 100 mg groups and -0.62 (-1.06 to -0.18) log₁₀ copies/ml between the entecavir 0.5 mg and lamivudine 100 mg groups, indicating the noninferiority of the entecavir 0.1 and 0.5 mg groups to the lamivudine 100 mg group and the superiority of the entecavir 0.5 mg group to the lamivudine 100 mg group ($P = 0.007$) (Table 2). In contrast, the entecavir 0.01 mg group was significantly inferior to the lamivudine 100 mg group (estimated mean difference = 1.20 [0.69 – 1.71]; $P < 0.0001$) (Table 2).

The secondary efficacy end point of a reduction in serum HBV DNA level 2 log₁₀ copies/ml or more or HBV DNA level less than 400 copies/ml by PCR assay was achieved

by 88.6% of patients in the entecavir 0.01 mg group and by 100% of patients in the entecavir 0.1 and 0.5 mg groups at week 22. Ninety-seven percent of patients in the lamivudine 100 mg group achieved this end point at week 22. HBV DNA level less than 0.7 MEq/ml by bDNA assay was achieved by 65.7%, 94.1%, and 100% of patients in the 0.01, 0.1, and 0.5 mg entecavir groups, respectively, and by 93.9% of patients in the lamivudine 100 mg treatment group.

Serologic response

Among HBeAg-positive patients, there was no significant difference between seroconversion rates at week 22 for the entecavir 0.01, 0.1, and 0.5 mg treatment groups (10.0%, 13.3%, and 3.6%, respectively) versus the lamivudine 100 mg treatment group (3.3%; Table 2). All patients who lost HBeAg also experienced HBeAg seroconversion.

Biochemical response

At baseline, elevated serum ALT levels ($>1.25 \times \text{ULN}$) were present in more than 90% of patients in all four treatment groups. At week 22, normal serum ALT levels (World Health Organization grade 0, $<1.25 \times \text{ULN}$) were recorded in similar proportions of patients in the entecavir 0.01, 0.1, and 0.5 mg treatment groups (75.0%, 85.3%, and 80.0% of patients, respectively) and the lamivudine treatment group (78.1% of patients), with no significant inter-group difference (Table 2).

Response

Response (HBV DNA level <0.7 MEq/ml by bDNA assay, HBeAg loss, and serum ALT level $<1.25 \times \text{ULN}$ for HBeAg-positive patients and HBV DNA level <0.7 MEq/ml by bDNA assay and serum ALT $<1.25 \times \text{ULN}$ for HBeAg-negative patients) was achieved by 14.3%, 20.6%, and 15.6% of patients in the entecavir 0.01, 0.1, and 0.5 mg

Table 2 Differences in HBV DNA levels between entecavir dose groups by PCR at week 22 in evaluable subjects

	0.1 mg ETV–0.01 mg ETV ($n = 34$, $n = 35$)	0.5 mg ETV–0.01 mg ETV ($n = 32$, $n = 35$)	0.5 mg ETV–0.1 mg ETV ($n = 32$, $n = 34$)
Estimated difference ^a (log ₁₀ copies/ml)	–1.61	–1.95	–0.23
Standard error	0.24	0.24	0.19
95% Confidence interval ^b	–2.20, –1.02	–2.53, –1.37	–0.69, 0.23
<i>P</i> -value	<0.0001	<0.0001	0.227

^a Estimated differences are regression-adjusted for baseline serum HBV DNA and HBeAg status

^b 95% Confidence interval is adjusted by modified Bonferroni procedures

ETV entecavir

Table 3 Virology and biochemical responses at week 22 and comparison of entecavir treatment groups with lamivudine in evaluable subjects

Response	ETV 0.01 mg (<i>n</i> = 35)	ETV 0.1 mg (<i>n</i> = 34)	ETV 0.5 mg (<i>n</i> = 32)	LVD 100 mg (<i>n</i> = 33)
HBV DNA by PCR assay				
Reduction from baseline at week 22 (log ₁₀ copies/ml), mean ± S.E.	−3.11 ± 0.18	−4.77 ± 0.17	−5.16 ± 0.13	−4.29 ± 0.18
HBV DNA estimated difference ^a (vs. LVD) (log ₁₀ copies/ml)	1.20	−0.39	−0.62	–
Standard error	0.26	0.22	0.22	–
95% Confidence interval	0.69, 1.71	−0.83, 0.05	−1.06, −0.18	–
<i>P</i> -value	<0.0001 ^b	0.081	0.007 ^c	–
HBV DNA by Roche Amplicor TM PCR assay				
Change in log ₁₀ HBV DNA reduction >2 or HBV DNA <400 copies/ml at week 22, <i>n</i> (%)	31 (88.6)	34 (100)	32 (100)	32 (97.0)
<i>P</i> -value (vs. LVD)	0.206	NR ^d	NR ^d	–
HBV DNA by Quantiplex assay				
HBV DNA <0.7 MEq/ml (2.5 pg/ml) at week 22, <i>n</i> (%)	23 (65.7)	32 (94.1)	32 (100)	31 (93.9)
<i>P</i> -value (vs. LVD)	0.002	1.000	NR ^d	–
Normalization of ALT levels ^e				
At week 22, <i>n</i> / <i>n</i> with abnormal baseline (%)	24/32 (75.0)	29/34 (85.3)	24/30 (80.0)	25/32 (78.1)
<i>P</i> -value (vs. LVD)	0.842	0.439	0.880	–
Loss of HBeAg and seroconversion at week 48 ^f				
HBeAg loss, <i>n</i> / <i>n</i> HBeAg positive at baseline (%)	3/30 (10.0)	4/30 (13.3)	1/28 (3.6)	1/30 (3.3)
HBeAg seroconversion	3/30 (10.0)	4/30 (13.3)	1/28 (3.6)	1/30 (3.3)
<i>P</i> -value (vs. LVD)	0.605	0.350	1.000	–
Response ^g at week 22, <i>n</i> (%)	5 (14.3)	7 (20.6)	5 (15.6)	3 (9.1)
<i>P</i> -value (vs. LVD)	0.735	0.190	0.480	–

^a Estimated differences are regression-adjusted for baseline HBV DNA and HBeAg status^b Two-sided test indicates inferiority of the entecavir 0.01 mg dose^c Two-sided test indicates superiority of the entecavir dose^d Not reported because expected counts <5^e WHO grade 0, ALT <1.25 × upper limit of normal^f Seroconversion was defined as disappearance of HBe-antigen and appearance of HBe-antibody^g Response was defined as HBV DNA levels <0.7 MEq/ml, HBeAg negativity and ALT <1.25 × ULN for HBeAg-positive patients and HBV DNA levels <0.7 MEq/ml and ALT <1.25 × ULN for HBeAg-negative patients

ETV entecavir

LVD lamivudine

treatment groups, respectively, and by 9.1% of patients in the lamivudine treatment group at week 22, and there were no significant differences in the rates of response between the four treatment groups (Table 2).

Resistance analysis

During the treatment period, serum HBV DNA level increased by 1 log₁₀ copies/ml or more from its nadir in one patient in the entecavir 0.01 mg group and one patient in the lamivudine 100 mg group. Nucleotide sequence analysis of the DNA polymerase coding region, using viral samples collected from these two patients at day 1 and at week 22, revealed no lamivudine-resistance substitutions

(rt180 and rt204 amino acid residues) [17, 18] or entecavir-resistance substitutions (rt184, rt202, and rt250 amino acid residues) [19].

Safety

During the study, adverse events were experienced by similar proportions of patients in the entecavir 0.01, 0.1, and 0.5 mg groups and the lamivudine 100 mg treatment group (97.1%, 97.1%, 91.2%, and 100.0%, respectively). Most adverse events were of mild or moderate intensity (grade 1/2) and transient. The most frequently reported adverse events (affecting ≥ 10% of patients in any one treatment group) included nasopharyngitis, headache, and

Table 4 Summary of adverse events and laboratory abnormalities during the 24-week blinded treatment phase

	ETV 0.01 mg (<i>n</i> = 35)	ETV 0.1 mg (<i>n</i> = 34)	ETV 0.5 mg (<i>n</i> = 34)	LVD 100 mg (<i>n</i> = 34)
Any adverse events	34 (97)	33 (97)	31 (91)	34 (100)
Most frequent clinical adverse events, ^a <i>n</i> (%)				
Nasopharyngitis	9 (25.7)	10 (29.4)	11 (32.4)	10 (29.4)
Headache	6 (17.1)	7 (20.6)	2 (5.9)	7 (20.6)
Diarrhea	1 (2.9)	1 (2.9)	4 (11.8)	4 (11.8)
Grade 3/4 clinical adverse events, <i>n</i> (%)	0	0	1 (2.9)	1 (2.9)
Grade 3/4 laboratory adverse events, <i>n</i> (%)	2 (5.7)	4 (11.8)	2 (5.9)	4 (11.8)
Any serious adverse events, <i>n</i> (%)	0	1 (2.9)	2 (5.9)	1 (2.9)
Discontinuations due to adverse events, ^b <i>n</i> (%)	0	0	1 (2.9)	1 (2.9)
ALT flares, ^c <i>n</i> (%)	0	1 (2.9)	1 (2.9)	2 (5.9)
Death, <i>n</i> (%)	0	0	0	0

^a Occurring in at least 10% of patients^b One patient treated with ETV 0.5 mg discontinued the study drug due to hepatic cirrhosis. One patient treated with lamivudine discontinued due to increased ALT^c ALT flare defined ALT >2 × baseline and 10 × ULN

ETV entecavir

LVD lamivudine

diarrhea (Table 4). Grade 3/4 clinical adverse events occurred in one patient in the entecavir 0.5 mg group (colon carcinoma) and one patient in the lamivudine group (anal ulcer); neither of these events was considered to be related to the study drug. Serious adverse events were limited to the above-mentioned case of colon carcinoma, serum ALT elevation (entecavir 0.1 mg group [*n* = 1], entecavir 0.5 mg group [*n* = 1]), and serum aspartate aminotransferase (AST)/ALT elevation (lamivudine 100 mg group [*n* = 1]), but these were not considered to be causally related to the study drug and did not necessitate treatment discontinuation. Transient ALT flares (serum ALT >2 × baseline level and >10 × ULN) occurred in four patients (entecavir 0.1 mg group [*n* = 1], entecavir 0.5 mg group [*n* = 1], and lamivudine 100 mg group [*n* = 2]) and were associated with HBV DNA level decreases of 2 log₁₀ copies/ml or more. None of the ALT flares were associated with hepatic decompensation and serum ALT and AST levels recovered to less than 1.25 × baseline level on continuation of the study treatment.

Discussion

The global ETV-005 study reported that entecavir was superior to lamivudine at reducing viral load in nucleoside-naïve patients with CHB infection [15]. We conducted the present study, using an identical design to the ETV-005 study, to determine whether the findings from this earlier

study are applicable to Japanese patients. In keeping with the previous findings, our results indicate that entecavir produces a dose-related reduction in serum HBV DNA level (0.01 < 0.1 ≤ 0.5 mg) in nucleoside-naïve Japanese patients with CHB; the log dose–response curves for the reduction in serum HBV DNA level with entecavir in the two studies were similar, with estimated regression curve slopes of −1.24 (Japanese study) and −1.32 (global study). In addition, both studies demonstrated the noninferiority of the entecavir 0.1 mg group compared with the lamivudine 100 mg group and the superiority of the entecavir 0.5 mg group compared with the lamivudine 100 mg group. The demonstration of a dose–response relationship for entecavir and the superiority of the entecavir 0.5 mg dose over lamivudine confirm that the antiviral activity of entecavir in Japanese patients is similar to that observed in study ETV-005. In a previous study, Ono et al. [14] demonstrated that the in vitro potency of entecavir was up to 2,200 times greater than that of lamivudine. The results presented here substantiate these earlier in vitro data and confirm the greater potency of entecavir over lamivudine in patients with CHB.

Serum ALT normalization rates with entecavir 0.5 mg and lamivudine 100 mg (~80%) were higher in the present study than those reported in the ETV-005 study (entecavir 0.5 mg, 69.0%; lamivudine 100 mg, 59.1%) [15]. In keeping with previous findings [20, 21], the incidence of entecavir-associated serum ALT flares in Japanese patients was low. The serum ALT flares occurred against a background of 2 log₁₀ copies/ml or more reductions in serum

HBV DNA level, and serum ALT levels subsequently normalized without discontinuation of entecavir. Therefore, the serum ALT flare noted here may indicate recovery of the host's immune response arising from the reduction in HBV viral titer [22, 23]. ALT flares have been reported after the discontinuation of entecavir therapy [15, 16], thus necessitating long-term follow-up to identify possible posttreatment viral rebound.

In conclusion, the results of this dose-ranging study demonstrate a clear dose–response relationship for entecavir in terms of mean HBV DNA level reduction at week 22. Entecavir 0.5 mg was significantly more effective than lamivudine 100 mg in reducing HBV DNA levels in nucleoside-naïve Japanese adult patients with CHB. At this dose level, entecavir treatment resulted in serum HBV DNA levels of less than 400 copies/ml in 100% of patients and normalization of serum ALT levels in 80% of patients after 22 weeks. Moreover, entecavir 0.5 mg once daily was well tolerated and showed a comparable safety profile to lamivudine.

References

- Maddrey WC. Hepatitis B: an important public health issue. *J Med Virol* 2000;61:362–366. doi:10.1002/1096-9071(200007)61:3<362::AID-JMV14>3.0.CO;2-I
- Kao JH, Chen DS. The natural history of hepatitis B virus infection. In Lai CL, Locarnini S, editors. *Hepatitis B Virus*. London: International Medical Press; 2002. 161–172
- Lok AS, McMahon BJ. American Association for the Study of Liver Diseases (AASLD) practice guidelines. *Chronic Hepatitis B*. *Hepatology* 2007;45:507–539. doi:10.1002/hep.21513
- Rivkin A. A review of entecavir in the treatment of chronic hepatitis B infection. *Curr Med Res Opin* 2005;21:1845–1856. doi:10.1185/030079905X65268
- Wong DKH, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Ann Intern Med* 1993;119:312–323
- Allen MI, Deslauriers M, Andrews CW, Tipples GA, Walters KA, Tyrrell DL, et al. Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. *Lamivudine Clinical Investigation Group*. *Hepatology* 1998;27:1670–1677. doi:10.1002/hep.510270628
- Chang TT, Lai CL, Chien RN, Guan R, Lim SG, Lee CM, et al. Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2004;19(11):1276–1282. doi:10.1111/j.1440-1746.2004.03428.x
- Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004;351(15):1521–1531. doi:10.1056/NEJMoa033364
- Omata M. Treatment of chronic hepatitis B infection. *N Engl J Med* 1998;339:114. doi:10.1056/NEJM199807093390209
- Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008;2:263–283. doi:10.1007/s12072-008-9080-3
- Genovesi EV, Lamb L, Medina I, Taylor D, Seifer M, Innaimo S, et al. Efficacy of the carbocyclic 2'-deoxyguanosine nucleoside BMS-200475 in the woodchuck model of hepatitis B virus infection. *Antimicrob Agents Chemother* 1998;42:3209–3217
- Marion PL, Salazar FH, Winters MA, Colonno RJ. Potent efficacy of entecavir (BMS-200475) in a duck model of hepatitis B virus replication. *Antimicrob Agents Chemother* 2002;46:82–88. doi:10.1128/AAC.46.1.82-88.2002
- Colonno RJ, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, et al. Entecavir resistance is rare in nucleoside naïve patients with hepatitis B. *Hepatology* 2006;44:1656–1665. doi:10.1002/hep.21422
- Ono SK, Kato N, Shiratori Y, Kato J, Goto T, Schinazi RF, et al. The polymerase L528M mutation cooperates with nucleotide binding-site mutations, increasing hepatitis B virus replication and drug resistance. *J Clin Invest* 2001;107:449–455. doi:10.1172/JCI11100
- Lai CL, Rosmawati M, Lao J, Van Vlierberghe H, Anderson FH, Thomas N, et al. Entecavir is superior to lamivudine in reducing hepatitis B virus DNA in patients with chronic hepatitis B infection. *Gastroenterology* 2002;123:1831–1838. doi:10.1053/gast.2002.37058
- Chang TT, Gish RG, Hadziyannis SJ, Cianciara J, Rizzetto M, Schiff ER, et al. A dose-ranging study of the efficacy and tolerability of entecavir in lamivudine-refractory chronic hepatitis B patients. *Gastroenterology* 2005;129:1198–1209. doi:10.1053/j.gastro.2005.06.055
- Ling R, Mutimer D, Ahmed M, Boxall EH, Elias E, Dusheiko GM, et al. Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. *Hepatology* 1996;24:711–713. doi:10.1002/hep.510240339
- Tipples GA, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DLJ. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine in vivo. *Hepatology* 1996;24:714–717
- Tenney DJ, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L, et al. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to lamivudine. *Antimicrob Agents Chemother* 2004;48:3498–3507. doi:10.1128/AAC.48.9.3498-3507.2004
- Chang TT, Gish R, de Man R, Gadano A, Sollano J, Chao YC, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006;354:1074–1076. doi:10.1056/NEJMoa051285
- Sherman M, Yurdaydin C, Sollano J, Silva M, Liaw Y-F, Cianciara J, et al. Entecavir is superior to continued lamivudine for the treatment of lamivudine-refractory. HBeAg-positive chronic hepatitis B. *Gastroenterology* 2006;130:2039–2049
- Boni C, Bertolotti A, Penna A, Cavalli A, Pilli M, Urbani S, et al. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. *J Clin Invest* 1998;102:968–975. doi:10.1172/JCI3731
- Nair S, Perrillo RP. Serum alanine aminotransferase flares during interferon treatment of chronic hepatitis B: is sustained clearance of HBV DNA dependent on levels of pretreatment viremia? *Hepatology* 2001;34:1021–1026. doi:10.1053/jhep.2001.28459