INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

The Efficacy of Short-term Interferon-beta Therapy for Chronic Hepatitis C Patients with Low Virus Load

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Abstract

Objective The aim of this study was to elucidate the efficacy of short-term interferon (IFN) therapy for chronic hepatitis C patients with low virus load.

Methods The present study was a retrospective cohort study. Inclusion criteria were biopsy-proven chronic hepatitis, the serum hepatitis C virus (HCV) RNA level of less than 100 KIU/ml, IFN period of 8weeks or less. One hundred and eleven consecutive patients satisfied above criteria were treated with IFN-beta (dose: 6 MU, daily for 4, 6, or 8 weeks).

Results Background of clinical profiles were as follows: median (range) age=56 (20-73) years, male/female=64/47, genotype 1b/2a/2b=40/68/3, and median (range) HCV-RNA= 34 (4.5-81) KIU/ml. Out of 111, 64 patients (57.7%) had sustained viral response (SVR). Based on the difference of HCV genotype, the SVR rate was 47.5% (19/40) in genotype 1 and 63.3% (45/71) in genotype 2. In genotype 1, the SVR rate in patients treated with the 8-week-regimen was significantly higher than that in patients treated with the 4- or 6-week regimen. In contrast, in genotype 2, the SVR in patients treated with the 8-week regimen was not significantly different from that in patients treated with the 6-week regimen. None of the patients had severe IFN-related side effects.

Conclusions The 6 or 8-week regiment of IFN-beta therapy is one selection of therapy for chronic hepatitis C patients who have tended to have a SVR and who show IFN-related adverse events.

Key words: chronic hepatitis C, low virus load, interferon, sustained viral response

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Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-5). Moreover, novel long-acting formulations of IFN known as pegylated IFN induced higher eradication rate of hepatitis C virus (HCV) (6-8). However, IFN is expensive and has a number of serious side effects. Therefore, if the treatment period becomes shorter, it could be preferable.

Several predictive factors of sustained viral response (SVR) to IFN have been identified, and these include a short duration of disease, young age, absence of liver cirrhosis, low HCV-RNA levels, HCV genotype 2a and mutant type of nonstructural 5A region (9-15). Low dose IFN tends to eradicate HCV RNA in patients who had a low serum level of HCV-RNA. However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in patients. Thus, in this study we evaluated the duration of IFN therapy in order to eradicate HCV RNA in patients who had low serum levels of HCV-RNA.

Abbreviations: HCV: hepatitis C virus, IFN: interferon, SVR: sustained viral response

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Table 1. Clinical Characteristics before Interferon Therapy in Chronic Hepatitis C Patients*

Characteristics	(n=111)
Age (years old) †	56(20-73)
Male/female ‡	64/47
Liver histology (fibrosis, 1/2/3) ‡	60/25/26
HCV genotype(1b/2a/2b) ‡	40/68/3
HCV load (KIU/ml) †	34 (4.5-81)
AST (IU/L) †	56 (14-226)
ALT (IU/L) †	76 (15-434)

***ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; and HCV, hepatitis C virus.**

† Data are expressed as median(range).

‡ Data are number of patients.

Materials and Methods

Patients

A total of 111 consecutive chronic hepatitis C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 1997 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated alanine aminotransferase (ALT) elevation greater than the upper normal limits (ALT normal range: 12-50 IU/L) for more than six months; histological evidence of chronic hepatitis within one year of entry into the trial; positive serum HCVRNA; serum HCV RNA level of less than 100 KIU/ml or 1 Meq/ml; genotype 1b, 2a and 2b. We excluded from the study all of the patients: 1) with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) Leukocytes <3,000/mm^3, platelets <80,000/mm^3 and bilirubin >1.5 mg/ml before IFN therapy.

One hundred eleven patients received IFN at a dose of 6 million units (MU) of natural IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan) daily for 4, 6 or 8 weeks. In general, patients were treated with IFN for 8 weeks. Eleven patients treated for 4 weeks and thirty patients treated for 6 weeks were assigned by randomized controlled trial. We regarded sustained viral response (SVR) to therapy as clearance of HCV RNA by RT-nested PCR (16) or amplicor method (17) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of this clinical trial as well as the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

Blood testing

Blood samples were obtained just before IFN therapy and stored at -80℃. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (18).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay or RT-nested PCR. The lower detection limit of the qualitative assay is 100 copies/ ml. HCV genotype was examined by the PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (19).

Liver histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman needle

Table 2. Predictive Factors for SVR in Patients with HCV Genotype 1*

Factor	Category	Odds ratio	95% CI†	p value
Period of IFN therapy (week)	4 or 6/ 8	1/8.93	2.14-37.03	0.003
AST (IU/L)	<76/≥76	1/2.17	0.85-5.55	0.102
Sex	Man / Woman	1/0.56	0.16-2.00	0.367
ALT (IU/L)	<100/≥100	1/1.67	0.47-5.93	0.430
Liver histology (fibrosis)	1 /2,3,4	1/0.79	0.39-1.60	0.507
Age (years)	<50/ ≥50	1/0.80	0.23-2.79	0.726

***ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon and CI; confidence interval.**

with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination included more than six portal areas. Histopathological interpretations of these 3-to 4-μm thick sections were made independently by experienced liver pathologists (YA and HK) who had no clinical information or knowledge of chronological order of the biopsies in each pair. The biopsy specimens were scored according to the system of Desmet et al (20).

Statistical analysis

Independent factors that might have influenced SVR were studied using the logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, liver histology, biochemical factors (aspartate aminotransferase (AST), ALT before IFN therapy, and period of IFN therapy. Significance of trends in SVR based on periods of IFN therapy was determined Cochran-Armitage trend test. The SPSS software package (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase

Results

Patients' characteristics

Table 1 shows the characteristics of the 111 patients who received IFN therapy. A total of 40 patients showed HCV genotype 1 and the remaining 71 patients showed HCV genotype 2.

Efficacy of treatment

Out of 111, 64 patients (57.7%) had SVR. Based on the difference of HCV genotype, the SVR rate was 47.5% (19/40) in genotype 1 and 63.3% (45/71) in genotype 2. We then investigated the factors associated with SVR after termination of IFN. Univariate analysis in patients with genotype 1 identified the following one factor that influenced SVR when the period of IFN treatment was 8weeks (Table 2). As one factor was associated with SVR, we did not evaluate the multivariate analysis.

On the other hand, univariate analysis in patients with genotype 2 did not identify the factor that influenced SVR (Table 3). In genotype 2, the SVR in patients treated with

Table 3. Predictive Factors for SVR in Patients with HCV Genotype 2 *

Factor	Category	Odds ratio	95% CI†	p value
AST (IU/L)	<76 /≥76	1/2.21	0.80-6.14	0.126
Sex	Man / Woman	1/0.61	0.22-1.64	0.324
Period of IFN therapy (week)	4 or 6/ 8	1/1.63	0.57-4.69	0.361
ALT (IU/L)	<100/≥100	1/1.22	0.41-3.57	0.721
Age (years)	<50/ ≥50	1/0.80	0.23-2.79	0.726
Liver histology (fibrosis)	1 /2,3	1/0.88	0.54-1.70	0.876

***ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; and IFN, interferon and CI; confidence interval.**

Table 4. SVR Based on HCV Genotype and Administration Period of Interferon

HCV genotype	Administration period (week)		
	4W	6W	8W
Genotype 1†	0% (0/6)	33.3% (5/15)	73.7% (14/19)
Genotype 2†‡	40% (2/5)	60% (9/15)	66.7% (34/51)

***HCV indicates hepatitis C virus; and SVR, sustained virological response.**
† p <0.001 in genotype 1, p =0.32 in genotype 2 by Cochran-Armitage method
‡ Three patients had HCV genotype 2b. These three patients were treated for 8 weeks and all the patients showed SVR. Remaining patients had genotype 2a.

the 8-week regimen was similar statistically to that in patients treated with the 4- or 6-week regimen.

Table 4 shows the SVR based on the HCV genotype and period of IFN therapy. According to Cochran-Armitage method, the 8-week IFN therapy regimen was the best in order to eradicate HCV RNA in genotype 1. On the other hand, in genotype 2, the 6-week regimen was almost the same as the 8-week regimen.

Adverse events

Within one week after the initiation of treatment, flu-like symptoms appeared in all the patients. Pain in the joints or muscle occurred in 50 cases. However, none of the patients withdrew from this treatment due to IFN-related side effects.

Discussion

We have described the efficacy of short-term IFN-beta therapy for chronic hepatitis C patients with low virus load. The present study was limited by a retrospective cohort trial. However, several findings from the present study have direct implications for the short-term IFN treatment of CH patients with low virus load. First, HCV RNA was cleared in more than 50% patients. Second, no patients withdrew from the treatment due to IFN-related side effects. Okanoue et al reported that side effects occurred when the daily IFN dose was increased (21). However, in the 8-week study period, there were no serious side effects. Third, the 8-week regimen of IFN therapy was preferable to eradicate HCV RNA compared to the 4 or 6-week regimen in genotype 1. On the other hand, in genotype 2, SVR by the 6-week regimen of IFN therapy was not significantly different from SVR by the 8-week regimen. These results indicate that 1) in patients with genotype 1 and low virus load, the 8-week regimen of IFN was recommended as the first treatment, 2) in patients with genotype 2 and low virus load, the 6-week regimen of IFN was recommended as the first treatment. This result is likely in agreement with several previous clinical trials (22-26).

In patients with genotype 1b and a high load of HCV-RNA, the clearance rate of HCV-RNA is less than 10% by the usual 6-month course of IFN monotherapy. In these IFN-resistant patients, the eradication rate of HCV-RNA level is at most 20-50% by the latest prolonged IFN therapy, combination therapy of IFN/ribavirin or pegylated IFN administration.

At present, combined IFN and ribavirin therapy is the standard therapy for chronic hepatitis C patients with genotype 1b and a high load of HCV-RNA. Next, in our hospital SVR of the 24-week IFN regimen in patients with a low load of HCV-RNA was 50.9% (220/432) in genotype 1b, 79.9% (279/349) in genotype 2a, and 71.4% (45/63) in genotype 2b. These results indicate that SVR of the 24-week regimen was higher than that of the short term regimen in genotype 2. However, prolonged IFN therapy is often associated with various side effects. A lower total dose and shorter administration period of IFN would be preferable in terms of both cost and safety.

Fortunately, patients with low HCV-RNA levels tend to eradicate HCV RNA with a low dose of IFN. The present study indicates that short-term IFN-beta therapy has no severe side effects. Thus, short-term IFN therapy is recommended for the patients who tend to have a SVR and have IFN- induced adverse events.

Conclusion

We think that the 6 or 8-week regimen of IFN-beta therapy is one therapy selection for chronic hepatitis C patients who tend to have a SVR and have IFN-induced adverse events.

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Original Article

Changes in viral loads of lamivudine-resistant mutants during entecavir therapy

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Aim: Entecavir therapy is effective against lamivudine-resistant virus in patients with hepatitis B virus infection. We investigated viral load changes of YMDD mutant virus (rtM204I [YIDD sequence], rtM204V [YVDD]) in serial serum samples during entecavir treatment for lamivudine-resistant virus and determined changes in viral precore and core promoter mutants.

Methods: Nineteen patients were treated in randomized, double-blind phase II clinical trials of entecavir at 0.5 or 1.0 mg for breakthrough hepatitis due to lamivudine-resistant virus. Viral changes in YMDD mutants (rtM204I, rtM204V), amino acid changes in the polymerase reverse transcriptase region and precore/core promoter mutations at 52 weeks were determined in 18 patients.

Results: Changes in viral loads of rtM204I and rtM204V were similar. No differences in load changes were seen between the 0.5 and 1.0 mg groups. However, load changes for rtM204I alone were greater than those for the rtM204I + rtM204V mixed-type ($P = 0.042$, at both 40 and 52 weeks). Load changes in rtM204I and rtM204V with *G1896A* tended to be greater than those without. Moreover, *G1896A* was replaced by wild-type virus in two patients at 52 weeks.

Conclusion: RtM204I only or the existence of precore mutation was more sensitive to entecavir therapy against lamivudine-resistant virus.

Key words: entecavir, hepatitis B virus, lamivudine, precore, YMDD mutant

INTRODUCTION

THERAPY IN PATIENTS with hepatitis B virus (HBV) aims to limit or reverse progression of the disease through the sustained suppression of HBV replication.[1] Approved therapies for chronic HBV infection involving treatment with interferon (IFN) have a low sustained response rate, undesirable side-effects, and high cost.[2,3] Several studies have reported that lamivudine is more effective and less costly than IFN in suppressing HBV replication, and also improves transaminase levels and liver histology, and enhances the rate of loss of hepatitis B e antigen (HBeAg).[4–7] On long-term use, however, lamivudine has the potential to induce viral resistance, with associated increases in HBV-DNA and serum transaminases.[8–10]

Entecavir (ETV), a deoxyguanosine analog, is a potent and selective inhibitor of HBV replication, with *in vitro* potency 100- to 1,000-fold greater than that of lamivudine.[11,12] Human clinical trials have demonstrated the efficacy of ETV in the treatment of chronic HBV infections.[13,14] The potential for additional therapeutic benefits with ETV was indicated by a reduced frequency of hepatocellular carcinoma in the woodchuck model and a prolongation of life span in chronically infected animals.[15] Data on the *in vitro* efficacy of ETV against lamivudine-resistant HBV are limited,[12] but several clinical studies have demonstrated *in vivo* efficacy.[16,17]

A recent report described a rapid, highly sensitive and reproducible method for quantifying mutant HBV virus in lamivudine-treated patients.[18] Using a real-time polymerase chain reaction (PCR; LightCycler; Roche

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Diagnostics, Mannheim, Germany) with a ResonSense probe, this method detects as little as 0.01% of YMDD mutant DNA among 10^5–10^9 copies of wild-type DNA. However, few reports have appeared on viral load changes in YMDD mutant virus (rtM204I [YIDD sequence] and rtM204V [YVDD]) during treatment with ETV against lamivudine-resistant HBV infection.

Among these, one recent study reported that two patients for whom previous therapies (lamivudine or famciclovir, ganciclovir, foscarnet and lamivudine) had failed exhibited virological breakthrough while on ETV.[19] The efficacy of ETV in these cases was decreased and viral load changes of YMDD mutant virus were increased, specifically via new substitutions plus lamivudine substitution (rtL180M and rtM204V) in the reverse transcriptase (rt) domain. We were therefore interested to analyze mutations of the rt domain of HBV polymerase in patients who had received long-term (52 weeks) ETV therapy against lamivudine-resistant HBV infection.

During chronic HBV infection, natural seroconversion to antibody to HBeAg (anti-HBe) usually correlates with the resolution of viremia and clinical recovery. Mutation in the precore region (nucleotide [nt] 1896) is related to the absence of HBeAg secretion[20] and may enhance the stability of the secondary structure of pregenome encapsidation signals, ensuring perpetuation of viral replication and thus contributing to viral persistence.[21] Buckwold *et al.* showed that the HBV genome carrying core promoter mutations (nt G1762A and A1764T) influenced viral replication.[22] Cho *et al.*[23] and our group[24] reported that lamivudine therapy resulted in reversion from precore and core promoter mutants to wild-type, but that these mutants reappeared during prolonged therapy. However, it is unclear how ETV influences precore and core promoter mutants of lamivudine-resistant virus.

In this prospective study, we investigated viral load changes in YMDD mutant virus (rtM204I, rtM204V) during ETV therapy against lamivudine-resistant HBV infection. Furthermore, we also analyzed serial serum samples from patients with lamivudine resistance to determine viral precore and core promoter mutants during treatment with ETV.

PATIENTS AND METHODS

Patients

THE PATIENTS WERE 19 consecutive adult Japanese patients treated in phase II between June 2003 and December 2004 at the Department of Gastroenterology, Toranomon Hospital. At entry, all patients were being treated with lamivudine (100 mg/day) for chronic hepatitis due to HBV infection when the emergence of YMDD motif mutations indicated the development of breakthrough hepatitis. They had not received other nucleoside analog drugs before lamivudine. The study was a phase II randomized (1:1), double-blind trial of ETV by repeat oral administration at 0.5 mg or 1.0 mg for 12 months. They were switched from lamivudine directly to ETV without any break in administration. All patients were negative for hepatitis C serological markers. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki, and written informed consent to participate was obtained from all patients.

Blood tests and serum viral markers

Routine biochemical tests were performed using standard procedures before and at least once monthly during therapy. Hepatitis B surface antigen (HBsAg), HBeAg and anti-HBe were determined with radioimmunoassay kits (Abbot Diagnostics, Chicago, IL, USA) according to the manufacturer's instructions. Serum HBV-DNA level and DNA sequence samples were stored at −80°C until assay. Serum HBV-DNA was quantified using the Roche Amplicor HBV Monitor assay (Roche Diagnostics, Indianapolis, IN, USA), a PCR-based assay with a lower limit of detection of 400 copies of HBV-DNA/mL (2.6 log copy/mL).

Quantitation of lamivudine-resistant mutants by real-time amplification refractory mutation system PCR

DNA was extracted from 100 μL serum. The assay was performed using a sensitive, real-time PCR-based assay for the detection of lamivudine resistance-associated mutations in the presence of high levels of wild-type virus, as reported recently.[18,25] Briefly, this method is based on the amplification refractory mutation system (ARMS) PCR for the detection of single-base mutations[26] and uses the same ARMS primers, reactions and cycling conditions on the LightCycler. To prepare the standards (rt204M, rtM204I and rtM204V), the first PCR product amplified using the primers P1 and P2[27] was cloned into the plasmid vector pBluescript (Stratagene, La Jolla, CA, USA) as reported previously. The concentration of purified plasmids was based on absorbance at 260 nm (GeneQuant II; Amersham Pharmacia Biotech, Tokyo, Japan). The standards for real-time PCR were prepared by serial dilution of a plasmid of known concentration. DNA values of these mutants below the

lower limit of detection were expressed as 2.0 log copy, and those over the upper limit as 9.0 log copy. The selectivity of this assay was tested as described previously[18,25] using reactions containing 10^9 copies of wild-type DNA (rt204M) template and from 0 to 10^9 copies of mutant virus (rtM204I or rtM204V) template. Under these conditions, the mutant primers (for rtM204I and rtM204V) detected the number of copies of mutant template present within the range of 10^4–10^9 copies. Moreover, one primer (for rt204I or rt204V) detected the number of copies within the range of 10^4–10^9 copies (mixed with 10^9 copies of the other mutant virus [rtM204V DNA or rtM204I DNA], respectively). Total HBV-DNA levels were measured by real-time PCR as described previously.[18] Serum samples were assayed at 11 time points, namely before (baseline) and at 2, 4, 8, 12, 16, 20, 24, 32, 40 and 52 weeks after the start of ETV. Data for the time-dependent decline in viral load relative to baseline were log-transformed, and thus all results for quantitative HBV level are expressed as $\log_{10}$ copy.

Determination of nucleotide sequences of HBV-DNA

DNA was extracted from 100 μL serum. PCR reactions for detection of the rt region (nt 130–1161) of HBV-DNA were performed in two parts. The first and second PCR reactions for detection of the first part of the rt region were performed using primers BGF1 (sense; 5′-CTGTGGAAGGCTGGCATTCT-3′) and BGR2 (antisense; 5′- GGCAGGATAGCCGCATTGTG-3′), and PreS-BamH1 (sense; 5′-CTTGGGATCCAGAGCTACAGCAT GG-3′) and BR112 (antisense; 5′-TTCCGTCGACATA TCCCATGAAGTTAAGGGA-3′), respectively, under conditions of initial denaturation for 4 min, 35 cycles of amplification at 94°C for 1 min, 55°C for 2 min, 72°C for 3 min and final extension at 72°C for 7 min. The first and second PCR reactions for detection of the second part of the same region were performed using primer pairs B11F (sense; 5′-GGCCAAGTCTGTA CAACATC-3′) and B12R (antisense; 5′-TGCAGAGGTG AAGCGAAGTG-3′), and B11F and B14R (antisense; 5′-GATCCAGTTGGCAGCACACC-3′), respectively, under the same conditions. The amplified PCR products were used for direct sequencing. Measurement of sequences in the rt region was performed at three time points, namely at the start of lamivudine, start of ETV, and 1 year after the start of ETV therapy. Nucleotide sequences of the core promoter and precore regions were determined as described previously,[24] with measurements taken at the same three time points.

Statistical analysis

Data are expressed as mean ± SD. Differences between groups were examined for statistical significance using the χ^2 test and Mann–Whitney *U*-test where appropriate. A two-tailed *P*-value less than 0.05 was considered significant.

RESULTS

Viral load changes in lamivudine-resistant mutants during ETV therapy

OF THE 19 patients participating in the present study, 10 received ETV at 0.5 mg and nine at 1.0 mg. However, serum samples for one patient without HBeAg receiving 0.5 mg were not available, and this patient was excluded. Baseline characteristics of the remaining 18 patients in Table 1 show no significant differences between the groups.

Changes in viral loads of rtM204I and rtM204V were measured in 18 patients. At the start of ETV, the number of patients with detectable rtM204I alone, rtM204V alone and mixed-type (rtM204I and rtM204V) was seven, 0 and 11, respectively. RtL180M was detected in all but one patient (no. 18) at ETV baseline. Figure 1 shows mean log changes in the viral loads of rtM204I ($n = 18$) and rtM204V ($n = 11$) from baseline during the initial 52 weeks of ETV, with no differences seen in viral load changes for rtM204I and rtM204V in the two ETV groups. The low rate of decrease in changes in the viral loads of rtM204V in the 1.0 mg group was due to a lower viral load of baseline.

Two patient types were recognized, rtM204I alone and rtM204I + rtM204V mixed. Table 2 shows that there were no differences except for HBeAg status between these two groups at the start of ETV therapy. The rate of HBeAg positivity in the rtM204I + rtM204V mixed group was high. Moreover, there were no differences in the rates of histological improvement, ALT normalization and loss of HBeAg until 52 weeks of treatment. ALT flare (ALT levels > twofold of baseline levels and > 10-fold of upper limit for the normal range) was found in one patient (no. 18 in Table 3) in the rtM204I alone group and in one patient (no. 1) in the rtM204I + rtM204V mixed group until 52 weeks of treatment. However, in both patients, the ALT flare was transient and was associated with declining HBV-DNA.

Compared with baseline for ETV, one or two new amino acid substitutions (except for ETV resistance substitutions) in the rt region were shown in six patients (one in the rtM204I alone group and five in the

Table 1 Patient characteristics at the start of entacavir therapy for lamivudine-breakthrough hepatitis

	0.5 mg	1.0 mg
Total number	9	9
Sex (female/male)	1/8	1/8
Age (years)	37 (29–65)	39 (30–49)
Alanine aminotransferase (IU/L)	124 (64–347)	119 (52–251)
Liver histology (F1/F2/F3/F4/N)‡	6/0/3/0/0	6/1/1/0/1
Serum HBV-DNA§ (Amplicor; log copy/mL)†	7.5 (6.2–>7.6)	> 7.6 (7.2–>7.6)
HBeAg (positive/negative)	6/3	7/2
HBV genotype (A/C)	1/8	0/9
YMDD mutant type (I/V/Mix)¶	4/0/5	3/0/6

†Data are median (range).
‡Liver histology, as liver fibrosis assessed on a four-point scale: F0, no fibrosis; F1, periportal expansion; F2, portoportal septa; F3, portocentral linkage or bridging fibrosis; F4, cirrhosis; N, liver biopsy not performed.
§HBV-DNA levels were measured by Amplicor HBV Monitor assay. HBV-DNA values below the lower limit of detection are listed as 2.6 log copy/mL and those over the upper limit of detection as 7.6 log copy/mL.
¶YMDD mutant type: I, rtM204I; V, rtM204V; Mix, rtM204I + rtM204V.
HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

rtM204I + rtM204V mixed group) at 52 weeks. However, four patients in the rtM204I group were PCR negative at 52 weeks and it was difficult to compare the difference of amino acid substitutions between both groups. Figure 2 shows that changes in the viral load of mutants were greater for rtM204I alone than for rtM204I in the mixed-type patients (3.00 ± 0.91 *vs* 2.21 ± 0.63, $P = 0.042$, at 40 weeks; 2.99 ± 0.87 *vs* 2.23 ± 0.78, $P = 0.042$, at 52 weeks). However, changes in the viral load of mutants were greater for rtM204I alone than for rtM204V in the mixed-type patients, although the difference was not statistically significant (2.99 ± 0.87 *vs* 1.90 ± 1.51, $P = 0.070$, at 52 weeks). Changes in the viral load of rtM204I and rtM204V in patients with the rtM204I + rtM204V mixed type were similar.

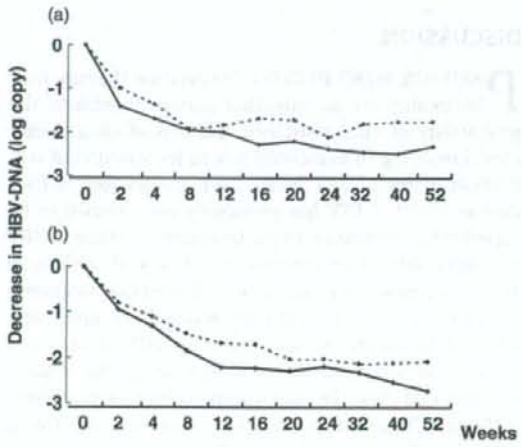

Figure 1 Mean log changes in viral loads of rtM204I and rtM204V from baseline during the initial 52-week treatment with entacavir at (a) 1.0 mg and (b) 0.5 mg. HBV-DNA levels of rtM204I (——) and rtM204V (-----) were measured by real-time polymerase chain reaction. HBV, hepatitis B virus.

Moreover, Table 3 shows precore sequences (nt 1896) at ETV baseline. Analysis of serum samples obtained at this time revealed a precore stop codon mutation (G1896A) in nine of 18 patients, among whom G1896A occurred as a mixed population with wild-type virus (G1896) in two and as a pure population in seven. Based on these findings, four groups were established by type of YMDD mutant and the presence of G1896A (rtM204I with G1896A [$n = 9$] and without G1896A [$n = 9$], and rtM204V with G1896A [$n = 4$] and without G1896A [$n = 7$]). Changes in the viral loads of rtM204I and rtM204V in these groups is shown in Figure 3; although patient numbers were small, changes tended to be greater in rtM204I and rtM204V with G1896A than in those without. Moreover, HBV-DNA levels in four patients (nos. 3, 16, 17 and 18) by Amplicor HBV Monitor assay were negative after 1 year of ETV therapy. YMDD motif in these patients was rtM204I only in all. Further, HBV-DNA levels in two additional patients (nos. 1 and 5) by Amplicor HBV Monitor assay were negative at 76 weeks of ETV therapy. These two patients had G1896A in the precore gene, although the YMDD

Table 2 Patient characteristics at the start of entacavir therapy in rtM204I alone and mix groups

	rtM204I	Mix (rtM204I + rtM204V)
Total number	7	11
Sex (female/male)	1/6	1/10
Age (years)†	37 (34–65)	39 (29–55)
Alanine aminotransferase (IU/L)†	119 (54–347)	112 (52–251)
Serum HBV-DNA‡ (Amplicor, log copy/mL)†	> 7.6 (6.2–>7.6)	> 7.6 (7.2–>7.6)
HBeAg (positive/negative)	3/4	10/1
HBV genotype (A/C)	0/7	1/9
Duration of lamivudine therapy (month)†	31 (19–47)	36 (10–48)

†Data are median (range).
‡HBV-DNA levels were measured by Amplicor HBV Monitor assay. HBV-DNA values below the lower limit of detection are listed as 2.6 log copy/mL and those over the upper limit of detection as 7.6 log copy/mL.
HBeAg, hepatitis B e antigen; HBV, hepatitis B virus.

motif type was mixed. Therefore, none of the seven patients with both the rtM204I + rtM204V mixed-type and precore wild-type showed a negative result on the Amplicor HBV Monitor assay at 76 weeks.

Entecavir-resistant mutant during therapy

Analysis of the rt region sequences (amino acids 1–344) of HBV polymerase in one patient (no. 11) at 52 weeks showed a new substitution of rtS202G in addition to the lamivudine substitutions (rtL180M and rtM204V), which may indicate introduced ETV resistance.[19] Virological rebounds of rtM204V viral load of this patient were observed at 40 and 52 weeks (increase of 1.27 and 1.08 log copies from nadir by real-time PCR). There were no patients with virological rebounds except this patient (no. 11).

Changes in precore and core promoter sequences before and during therapy

Precore and core promoter sequences in 18 patients were analyzed during 1 year of treatment with ETV for lamivudine-breakthrough hepatitis. Precore sequences at baseline for lamivudine were the same as those at baseline for ETV in 10 of 18 patients (excluding one lacking lamivudine baseline data; Table 3). Analysis of serum samples obtained at ETV baseline revealed a precore stop codon mutation (*G1896A*) in nine of 18 patients. After the start of ETV, *G1896A* was replaced by wild-type virus in two patients (nos. 1 and 5) at 1 year. However, G1896 was replaced by *G1896A* in one patient (no. 11) with ETV resistance. Thus, *G1896A* was observed in five of 14 patients, excluding four PCR-negative patients, at 1 year.

Core promoter sequences at baseline for lamivudine therapy were the same as those at baseline for ETV in 15 of 18 patients (Table 3). Among the 18, 15 had core promoter mutations (A1762T and G1764A) in samples collected at ETV baseline. During treatment, core promoter mutations at baseline were similar to those at 1 year.

YMDD mutant type was changed after 1 year of ETV treatment in three patients (nos. 1, 7 and 13), with the baseline rtM204I + rtM204V mixed types replaced by the respective major YMDD mutant.

DISCUSSION

PATIENTS WHO RECEIVE lamivudine therapy may be treated for an extended period, increasing the probability of viral resistance and loss of clinical efficacy, involving an associated risk of increased viral replication, flares of ALT levels, and progression of liver disease.[5,8–10,28, 29] ETV has previously been shown to be superior to lamivudine in the treatment of chronic HBV in nucleoside-naïve patients infected with wild-type HBV.[14] Moreover, a recent report showed that treatment with ETV at 0.5 or 1.0 mg daily was well tolerated, and resulted in significant reductions in HBV-DNA levels as well as normalization of ALT levels in HBeAg-positive and -negative lamivudine-refractory patients.[17] Although ETV in the recent report[17] was more effective at 1.0 than 0.5 mg, no differences between the two groups were seen in changes in viral load in the present study. Moreover, results for viral load changes by Amplicor HBV Monitor assay were the same in the present study (data not shown). This difference between these studies

Table 3 Serial precore and core promoter sequences of patients treated with lamivudine and entecavir

Patient	Genotype	Lamivudine						Entecavir								
		Baseline						Baseline						1 year		
		eAg	YMDD Motif	Precore nt 1896	CP nt 1762	CP nt 1764	YMDD eAg	Motif	Precore nt 1896	CP nt 1762	CP nt 1764	YMDD eAg	Motif	Precore nt 1896	CP nt 1762	CP nt 1764
1	C	+	M	G	A/T	G/A	+	V+I	G/A	T	A	+	V	G	T	A
2	C	+	M	G	T	A	+	V+I	G	T	A	+	V+I	G	T	A
3	C	+	M	G	T	A	+	I	G	T	A	+	N	N	N	N
4	C	+	M	G/A	A	G	+	V+I	A	A	G	+	V+I	A	A	G
5	C	ND	ND	ND	ND	ND	+	I+V	G/A	T	A	+	I+V	G	A	A
6	C	+	M	G	T	A	+	I+V	G	T	A	+	I+V	G	T	A
7	A	+	M	G	D	D	+	V+I	G	D	D	+	V	G	T	A
8	C	+	M	G	T	A	+	I	G	T	A	+	I	G	T	A
9	C	+	M	G/A	D	D	+	I+V	A	A/T	G/A	+	I+V	A	T	A
10	C	+	M	G	T	A	+	I+V	G	T	A	+	I+V	G	T	A
11	C	+	M	G	T	A	+	I+V	G	T	A	+	I+V	A	T	A
12	C	+	M	G	T	A	+	I+V	G	T	A	+	I+V	G	T	A
13	C	+	M	G/A	T	A	+	I+V	G	T	A	–	I	G	T	A
14	C	+	M	G/A	A	A	–	I	A	A	A	–	I	A	A/T	A
15	C	–	M	G/A	T	A	–	I	A	T	A	–	I	A	T	A
16	C	–	M	A	T	A	–	I	A	T	A	–	N	N	N	N
17	C	–	M	G/A	T	A	–	I	A	T	A	–	N	N	N	N
18	C	–	M	A	T	A	–	I	A	T	A	–	N	N	N	N

Baseline, time at the beginning of therapy; CP, core promoter; D, deletion; eAg, HBeAg; ND, not done; N, polymerase chain reaction-negative; YMDD motif: M, YMDD; I, rtM204I; V, rtM204V; I + V, mixed-type (rtM204I and rtM204V), major type is listed first.

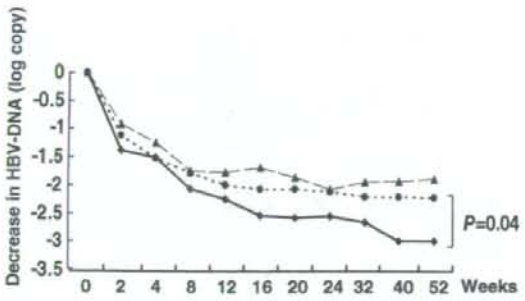

Figure 2 Mean log changes in the viral loads of each mutant of rtM204I alone and of rtM204I + rtM204V mixed type from baseline during the initial 52-week treatment with entacavir. HBV-DNA levels of rtM204I and rtM204V were measured by real-time polymerase chain reaction. HBV, hepatitis B virus. (◆), rtM204I in rtM204I alone; (●), rtM204I in mixed type; (▲), rtM204V in mixed type.

may have resulted from differences in race or HBV genotype (the major genotype is C in Japan). Few reports of ETV therapy against lamivudine-resistant HBV genotype C infection have appeared, and further study is necessary.

It has been reported that ETV is most effective against wild-type HBV (YMDD) and shows almost equally effective inhibition of the replication of rtM204I, rtL180M/rtM204V (rtM204V with rtL180M) and rtL180M/rtM204I (rtM204I with rtL180M) *in vitro*.[12,30] Supporting this, our study showed that rtM204I and rtM204V had similar sensitivity to ETV *in vivo*. In our study, however, rtM204I only or the presence of a precore mutation was more sensitive to ETV. These findings were seen mainly in patients without HBeAg. Moreover, rtM204I only or the presence of a precore mutation was also present in three HBeAg-positive patients (nos. 1, 3 and 5) whose HBV-DNA levels were negative on Amplicor HBV Monitor assay at 76 weeks. Our recent report also showed the greatest change in viral load was seen for rtM204I without HBeAg during adefovir dipivoxil (ADV) in addition to ongoing lamivudine therapy.[25] Considering these data, rtM204I virus is more sensitive to antiviral nucleoside analogs.

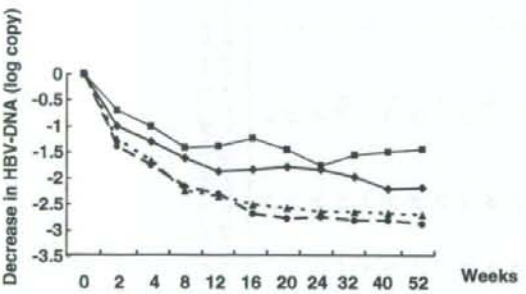

Figure 3 Mean log changes in the viral loads of rtM204I or rtM204V with or without *G1896A* from baseline during the initial 52-week treatment with entacavir. HBV-DNA levels of rtM204I and rtM204V were measured by real-time polymerase chain reaction. HBV, hepatitis B virus. (■), rtM204V without *G1896A*; (◆), rtM204I without *G1896A*; (▲), rtM204V with *G1896A*; (●), rtM204I with *G1896A*.

During lamivudine therapy, precore mutants tended to be replaced by wild-type virus at 1 year, and this was unrelated to the emergence of YMDD motif mutations.[23,24] Patients who showed mutations in the YMDD motif during long-term lamivudine therapy, in contrast, exhibited the reappearance of precore mutants.[24] Although the number of patients was small, ETV therapy in the present study also appeared to result in the preferential selection of wild-type virus. This finding was also seen from changes in the viral load of rtM204I and rtM204V with *G1896A* (Table 3). This finding suggests that antiviral nucleoside analogs such as lamivudine and ETV selectively suppress precore mutants over wild-type virus. In contrast, we did not see this replacement by wild-type at 1 year for core promoter mutations. Our results thus conflict with those of two previous studies, which showed that core promoter mutations during lamivudine therapy also tended to be replaced by wild-type virus at 1 year[23,24] and, more recently, that three of five seroconverters of HBeAg harbored core promoter mutations at baseline that were progressively replaced by the wild-type genome during ADV monotherapy.[31] The reason for this apparent discrepancy is unclear. In any case, our present study indicated that, compared to initial lamivudine therapy or ADV monotherapy, ETV may be less effective against core promoter mutants than wild-type virus.

In a recent investigation of lamivudine-resistant viruses, additional substitutions at rtT184, rtS202, or rtM250 were shown to further reduce ETV susceptibility.[19] The present and above studies add those at rtL180M and rtM204V to this list. One of our patients had rtS202G substitution in addition to rtL180M and rtM204V. Importantly, however, breakthrough hepatitis may occur even when the rate of substitutions (ETV-resistant) is not high.[19] Taken together, these findings suggest that ETV therapy may not be beneficial in

patients with either or both YVDD and no precore mutant.

In conclusion, we analyzed changes in viral loads of rtM204I and rtM204V during ETV therapy for lamivudine-resistant virus. Results showed that rtM204I and rtM204V had similar sensitivity to ETV. However, rtM204I only or the existence of a precore mutation conferred greater sensitivity to ETV. Moreover, antiviral nucleoside analogs such as lamivudine and ETV selectively tended to suppress precore mutants over wild-type virus. Further studies of virological changes and clinical efficacy during longer ETV therapy for lamivudine-resistant virus are necessary.

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HEPATOLOGY

Efficacy and safety of entecavir in lamivudine-refractory patients with chronic hepatitis B: Randomized controlled trial in Japanese patients

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chronic hepatitis B, entecavir, Japanese, lamivudine.

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Abstract

Background and Aim: Entecavir is a potent inhibitor of both wild-type and lamivudine-resistant hepatitis B virus (HBV) with proven clinical efficacy. We conducted a randomized, double-blind, multicenter study in Japan (ETV-052) evaluating the efficacy and safety of two doses of entecavir in adult patients with lamivudine-refractory chronic hepatitis B infection.

Methods: Eighty-four patients with chronic hepatitis B who were refractory to lamivudine therapy were switched from lamivudine to daily oral doses of 0.5 mg entecavir (41 patients) or 1 mg entecavir (43 patients) for 52 weeks.

Results: The proportions of patients achieving the primary end-point ($\geq$2 $\log_{10}$ reduction in HBV-DNA from baseline by polymerase chain reaction assay or undetectable HBV-DNA levels [<400 copies/mL] at week 48) were 90% and 93% for entecavir 0.5 mg and 1 mg, respectively, with 33% of patients in each dosing group achieving <400 copies/mL. The mean reduction in HBV-DNA from baseline was 3.58 and 3.75 $\log_{10}$ copies/mL for entecavir 0.5 mg and 1 mg, respectively. High proportions of patients achieved alanine aminotransferase normalization at week 48 (0.5 mg 86%, 1 mg 78%). Histological improvement was observed in most patients (0.5 mg 52%, 1 mg 60%). Virological breakthrough (increase in HBV-DNA of $\geq$1 $\log_{10}$ copies/mL from nadir) was observed in one patient but was not associated with selection of entecavir-associated resistance substitutions. Entecavir was well tolerated, with no patients discontinuing study drug due to adverse events.

Conclusions: These findings indicate that entecavir is safe and effective for the treatment of Japanese adults with lamivudine-refractory chronic hepatitis B.

Introduction

Chronic hepatitis B is a life-threatening disease, often called the 'silent killer', which eventually leads to liver cirrhosis, decompensated hepatic disease, or hepatocellular carcinoma in 20–40% of patients.[1] Prevalence is high in the Asia–Pacific region; of the 350 million patients with chronic hepatitis B worldwide, approximately 75% live in Asia.[2] Since the introduction of mass vaccination in Japan, hepatitis B virus (HBV) infection rates have dropped significantly (0.8% in 2000); however, 38% of these individuals are chronically infected and the disease burden is still high.[2] Recent studies indicate that elevated levels of HBV-DNA are associated with the development of cirrhosis and hepatocellular carcinoma and that the risk of complications and disease progression can be reduced by suppressing HBV-DNA replication with antiviral therapy.[3–5] However, the emergence of resistance and the consequent re-elevation in viral loads negates the benefit of therapy[6] and alternative treatment options are required for further benefit.

Lamivudine was the first nucleoside analog developed for the treatment of chronic hepatitis B and was approved by the FDA in 1998. Since then, this drug has been widely used because of its efficacy during short-term dosing;[5,7] however, relapse of hepatitis associated with the emergence of lamivudine-resistant viral strains has proved to limit its long-term efficacy.[6,8,9] The incidence of resistance has been reported to be approximately 20% annually.[6,8,9]

Lamivudine-resistant viruses carry substitutions in the reverse transcriptase region of HBV polymerase, usually a methionine to valine or isoleucine at amino acid position 204 (rtM204I/V) in the tyrosine–methionine–aspartate–aspartate (YMDD) motif which is often accompanied by a compensatory substitution at position 180 (rtL180M). Lamivudine resistance leads to loss of virological suppression and increases in serum alanine aminotransferase (ALT) levels, which may be followed by hepatitis progression and, in patients with cirrhosis, by hepatic decompensation, hepatocellular carcinoma, and removal from liver transplant lists.[10,11] Continued lamivudine treatment is of no clinical benefit in these patients, so alternative treatments are needed.[6] Adefovir dipivoxil has demonstrated activity against lamivudine-resistant virus, with increasing evidence that adding adefovir to continued lamivudine is associated with lower adefovir resistance rates.[12,13]

Entecavir is a deoxyguanosine analog that has recently been approved in many countries worldwide, including Japan. Entecavir exhibits potent antiviral activity, with a 50% effective concentration more than 300-fold greater than that of lamivudine *in vitro*.[14,15] In multinational phase II and III clinical trials, entecavir has been shown to be well tolerated and effective for the treatment of chronic hepatitis B in lamivudine-refractory patients at a dose of 1 mg daily.[14,16,17] We report here the results of a phase II clinical trial assessing the efficacy and safety of 52 weeks of entecavir treatment in Japanese patients with lamivudine-refractory chronic hepatitis B.

Methods

Patients

The present study included men and women, aged 20–75 years, with chronic hepatitis B infection and evidence of active viral replication (HBV-DNA levels $>10^5$ copies/mL) despite ongoing lamivudine therapy, with a history of at least 24 weeks of lamivudine therapy or documented evidence of infection with HBV carrying lamivudine-associated substitutions. Patients were required to have elevated ALT levels (1.3 to 10× the upper limit of normal [ULN]), compensated liver disease (total bilirubin ≤2.5 mg/dL, prothrombin time ≤3 s longer than normal control value or international normalized ratio ≤1.5, albumin ≥3.0 g/dL, and no current evidence or history of variceal bleeding, hepatic encephalopathy, or ascites requiring diuretics or paracentesis).

Exclusion criteria included the following: coinfection with hepatitis C virus, hepatitis D virus, or human immunodeficiency virus; other forms of liver disease; therapy with any anti-HBV drug other than lamivudine within 24 weeks prior to randomization; and more than 12 weeks of therapy with a nucleoside or nucleotide analog with activity against HBV.

Written informed consent was obtained from all subjects, and the study was conducted in compliance with the Declaration of Helsinki, Good Clinical Practice Guidelines, and Articles/Notifications of the Ministry of Health and Labor in Japan.

Study design

This was a multicenter, randomized, double-blind study carried out at 16 sites in Japan. Eligible subjects were randomly assigned to receive either entecavir 0.5 mg daily ($n = 41$) or entecavir 1 mg daily ($n = 43$) for 52 weeks. Patients continued their lamivudine therapy until the start of entecavir treatment. Patients who completed 52 weeks of dosing could enroll in an open-label entecavir rollover study. Patients not entering the rollover study were to be followed up for 24 weeks post-dosing and could receive alternative anti-HBV therapy.

Study assessments and end-points

Response to therapy was assessed at Week 52, based on results obtained at Week 48. Patients were seen for scheduled visits at baseline (Day 1), Weeks 2 and 4, and every 4 weeks until Week 52 or the end of dosing. HBV-DNA levels were determined by Roche Amplicor™ polymerase chain reaction (PCR) assay (lower limit of detection 400 copies/mL)[18] at baseline and at weeks 4, 8, 12, 24, 36, and 48. Histological examinations were carried out for all patients via liver biopsy at baseline and at Week 48. Biopsies were evaluated by a Biopsy Reading Committee blinded to treatment and sequence, and were scored according to the Knodell histological activity index (HAI) and fibrosis scores,[19] and the corresponding New Inuyama scores.[20]

The primary efficacy end-point was the proportion of subjects achieving a reduction in HBV-DNA by PCR assay of at least 2 $\log_{10}$ copies/mL from baseline or to <400 copies/mL at Week 48. Secondary end-points included the mean change from baseline in HBV-DNA and the proportions of patients achieving HBV-DNA <400 copies/mL; ALT <1.25× ULN; hepatitis B e antigen (HBeAg) loss and HBeAg seroconversion (loss of HBeAg and appearance of antibody to HBeAg [anti-HBe]) among patients who were HBeAg positive at baseline; and complete response (defined as HBV-DNA <400 copies/mL by PCR assay and ALT <1.25× ULN and being HBeAg negative if they were HBeAg positive at baseline) at Week 48. Histological improvement was defined as a ≥2-point decrease in Knodell necroinflammatory score and no worsening of fibrosis (i.e. ≥1-point increase in Knodell fibrosis score). Liver biopsies were also evaluated using the New Inuyama classification system.

Safety information was obtained from all patients who received at least one dose of study drug. Safety end-points included adverse events, laboratory abnormalities, and discontinuation of study drug due to adverse events or laboratory abnormalities. The date of onset, measures taken, causes, and outcome of all adverse drug reactions were investigated and recorded. Causal relationships with the study drug were assessed by the physician. The proportion of patients who experienced an ALT flare (defined as an on-treatment ALT measurement >2× baseline and >10× ULN) was also determined.

Resistance assessment

Genotypic analysis to detect substitutions in the HBV polymerase at residues rt180, 184, 202, 204, and 250 was carried out on HBV-DNA serum samples collected at Week 24 (or last available on-therapy sample for patients who discontinued prematurely) for all patients who experienced a ≥1 $\log_{10}$ copies/mL increase in HBV-DNA from the nadir. Genotypic resistance analysis of HBV-DNA polymerase was performed at a central laboratory (SRL, Inc., Tokyo, Japan). HBV-DNA was extracted and codons 1 to 344 of the reverse transcriptase encoding region were PCR amplified

and sequenced at a central laboratory. We also investigated lamivudine resistance-associated substitutions (at rt204) using a PCR-enzyme-linked minisequence assay (Medical & Biological Laboratories Co., Aichi, Japan) in all patients at baseline and at Week 48.

Statistical analysis

Analyses of efficacy end-points were based on treated subjects. For binary end-points, subjects with missing Week 48 measurements were treated as missing (non-completer = missing analysis). Ninety-five percent confidence intervals (CI) were calculated for proportions of patients achieving each end-point. Parameters represented by continuous variables were summarized by the mean and standard error. Wilcoxon signed-rank test was carried out for comparison of New Inuyama scores in baseline and post-treatment samples.

Results

Study population

Of the 115 patients enrolled and screened, 84 were randomized and received at least one dose of study treatment. Forty-one patients received entecavir 0.5 mg and 43 received entecavir 1 mg daily (Table 1). One patient in each group withdrew consent before completing 48 weeks of treatment. All remaining patients completed 52 weeks of dosing (Table 1) and then entered a rollover study. The two treatment arms were well balanced at baseline for demographic and disease characteristics (Table 1). Most patients were male (85%) and all were Japanese, with a mean age of 44.1 years in the entecavir 0.5 mg group and 42.3 years in the entecavir 1 mg group. The majority of patients were HBeAg positive (73% entecavir 0.5 mg, 77% entecavir 1 mg) and were infected with HBV genotype C (90.0% entecavir 0.5 mg, 97.6% entecavir 1 mg). Mean HBV-DNA at baseline was 7.72 $\log_{10}$ copies/mL in the 0.5 mg group and 7.59 $\log_{10}$ copies/mL in the 1 mg group. Virus from all patients carried YMDD substitutions at rt204. No patients had previously received interferon therapy.

Table 1 Disposition and baseline characteristics of lamivudine-refractory chronic hepatitis B patients treated with entecavir

	Entecavir 0.5 mg	Entecavir 1 mg
Randomized	41	43
Treated	41	43
Completed 52 weeks of dosing	40	42
Male, *n* (%)	35 (85)	37 (86)
Age (years), mean ± SD	44.1 ± 10.5	42.3 ± 7.3
Weight (kg), mean ± SD	67.6 ± 10.7	65.7 ± 11.2
Ethnicity		
Japanese, *n* (%)	40 (100)	42 (100)
HBV-DNA, mean ± SD		
$\log_{10}$ copies/mL by PCR	7.72 ± 0.77	7.59 ± 1.07
HBeAg positive, *n* (%)	30 (73)	33 (77)
ALT (IU/L), mean ± SD	134.5 ± 119.8	132.9 ± 102.1
Knodell HAI score		
Mean ± SE (*n*)	7.2 ± 0.6 (26)	6.5 ± 0.5 (35)
YMDD mutation present	41 (100)	43 (100)
HBV genotype, *n* (%)		
C	36 (90.0)	41 (97.6)
A	1 (2.5)	0
B	1 (2.5)	1 (2.4)
Others (D, E, F, AD, DE)	2 (5.0)	0

ALT, alanine aminotransferase; HAI, histological activity index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction; YMDD, tyrosine–methionine–aspartate–aspartate motif.

Virological response

A reduction in HBV-DNA by 2 $\log_{10}$ copies/mL or to below the limit of detection (400 copies/mL) at Week 48 (the primary end-point) was achieved by 90% (95% CI 76.3, 97.2) of patients in the entecavir 0.5 mg group and 93% (95% CI 80.5, 98.5) of patients in the entecavir 1 mg group (Table 2). In the entecavir 1 mg group, this end-point was achieved by 74% of patients by Week 4 and by more than 90% of patients by week 8 (Fig. 1). In the entecavir 0.5 mg treatment group, 24 weeks of treatment were required for 90% of patients to achieve the end-point. Similarly, a higher proportion of patients in the 1 mg group than in the 0.5 mg group achieved undetectable levels of HBV-DNA (<400 copies/mL) at Week 24 (19% [8/42 patients] *vs* 13% [5/40 patients] respectively), but the proportions of patients achieving this end-point were similar by Week 48 (33% [14/42 patients] *vs* 33% [13/40 patients] respectively).

Entecavir treatment resulted in a rapid decline in HBV-DNA levels in both treatment groups (Fig. 2), achieving a mean change from baseline at Week 24 of -3.20 ± 0.18 $\log_{10}$ copies/mL for the 0.5 mg group and -3.44 ± 0.18 $\log_{10}$ copies/mL for the 1 mg group (Table 2). Further reductions were observed between Weeks 24 and 48, with the mean change from baseline reaching -3.58 ± 0.21 $\log_{10}$ copies/mL and -3.75 ± 0.19 $\log_{10}$ copies/mL in the entecavir 0.5 and 1 mg groups, respectively (Fig. 2).

Biochemical response

At baseline, approximately 90% of patients exhibited ALT levels ≥1.25× ULN. The proportion of subjects whose ALT levels had normalized (<1.25× ULN: WHO toxicity grade 0) at Week 24 was 76% (28/37 patients) for the 0.5 mg group and 73% (27/37 patients) for the 1 mg group. At Week 48, the proportions increased to 86% (32/37 patients) for the 0.5 mg group and 78% (29/37 patients) for the 1 mg group (Table 2). The differences between the two groups were not significant at either time point ($P = 0.422$).

Serological response

Among HBeAg-positive patients at baseline, the proportion of patients who achieved HBeAg seroconversion (loss of HBeAg and appearance of anti-HBe) was higher in the 1 mg group at Week 24 (9% [3/33 patients] compared with 0% [0/29 patients] for the 0.5 mg group) but was similar between groups at Week 48; 17% (5/29 patients) for the 0.5 mg group and 15% (5/33 patients) for the 1 mg group ($P = 1.000$; Table 2).

Table 2 Virological, biochemical, and histological responses at Weeks 24 and 48

	Entecavir 0.5 mg ($n = 40$)	Entecavir 1 mg ($n = 42$)
HBV-DNA by Roche Amplicor™ PCR assay		
Reduction in HBV-DNA >2.0 $\log_{10}$ copies/mL or undetectable at Week 48 (primary study end-point), *n* (%)	36 (90)	39 (93)
Reduction from baseline ($\log_{10}$ copies/mL), mean ± SE		
At Week 24	−3.20 ± 0.18	−3.44 ± 0.18
At Week 48	−3.58 ± 0.21	−3.75 ± 0.19
HBV-DNA <400 copies/mL, *n* (%)		
At Week 24	5 (13)	8 (19)
At Week 48	13 (33)	14 (33)
Normalization of ALT levels†		
At Week 24, *n*/*n* with abnormal baseline (%)	28/37 (76)	27/37 (73)
At Week 48, *n*/*n* with abnormal baseline (%)	32/37 (86)	29/37 (78)
HBeAg seroconversion‡		
At Week 24, *n*/HBeAg positive *n* at baseline (%)	0/29 (0)	3/33 (9)
At Week 48, *n*/HBeAg positive *n* at baseline (%)	5/29 (17)	5/33 (15)
Complete response§ at Week 48, *n* (%)	6 (15)	6 (14)
Histological improvement		
Histological improvement,¶ *n* (%)	12/23†† (52)	21/35†† (60)
Knodell HAI scores, reduction from baseline at Week 48, mean ± SE	−3.3 ± 0.6*	−3.6 ± 0.5*
Improvement of grading score in Inuyama classification, *n* (%)	16/26 (62)*	19/35 (54)*

*$P < 0.0001$ compared with baseline, Wilcoxon signed-rank test.
†WHO grade 0, ALT <1.25× ULN.
‡Seroconversion was defined as the acquisition of antibodies to HBeAg.
§Complete response was defined as undetectable HBV-DNA (<400 copies/mL), HBeAg negative, and normal ALT.
¶≥2-point decrease in Knodell necroinflammatory score from baseline with no worsening of the Knodell fibrosis score.
††Patients with evaluable baseline and Week 42 history.
ALT, alanine aminotransferase; HAI, histological activity index; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction; ULN, upper limit of normal.

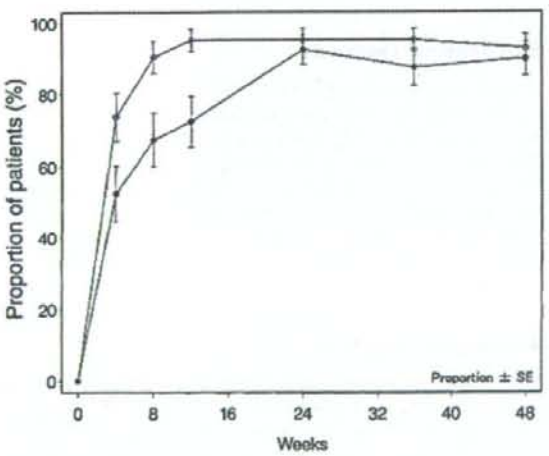

Figure 1 Proportion of patients (%) who achieved a reduction in hepatitis B virus (HBV)-DNA ≥2.0 $\log_{10}$ copies/mL or <400 copies/mL in the entecavir 1 mg (○) and 0.5 mg (●) groups.

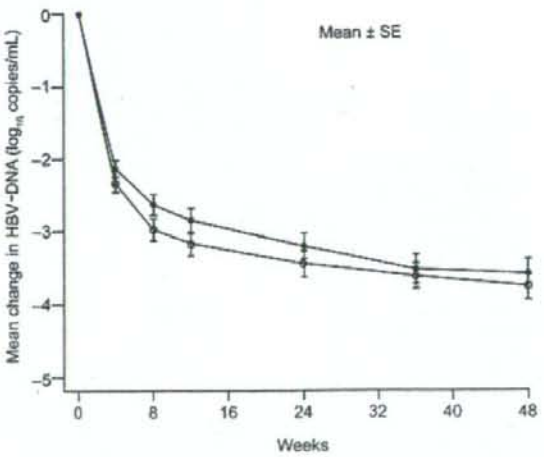

Figure 2 Mean change from baseline in hepatitis B virus (HBV)-DNA ($\log_{10}$ copies/mL) by polymerase chain reaction assay through 48 weeks in patients treated with entecavir 1 mg (○) and 0.5 mg (●).

Table 3 Summary of adverse events reported during treatment periods

	No. subjects (%)	
	Entecavir 0.5 mg ($n = 41$)	Entecavir 1 mg ($n = 43$)
Any adverse event	41 (100)	43 (100)
Clinical adverse event	37 (90)	40 (93)
Laboratory adverse event	38 (93)	38 (88)
Most frequent clinical adverse events†		
Nasopharyngitis	15 (37)	21 (49)
Headache	9 (22)	13 (30)
Upper respiratory tract infection	7 (17)	1 (2)
Malaise	6 (14.6)	9 (21)
Any severe (grade 3–4) adverse events‡ (%)	6 (15)§	5 (12)¶
Any serious adverse events‡ (%)	1 (2)	3 (7)
Discontinuations due to adverse events‡ (%)	0	0
Death (%)	0	0
ALT flare††	0	3 (7)

†Occurring in at least 15% of patients.
‡Including laboratory abnormalities.
§Eight events occurred in six subjects.
¶Nine events occurred in five subjects.
††ALT flare defined as ALT >2× baseline and 10× ULN. All ALT flares were associated with ≥2 $\log_{10}$ reduction in HBV-DNA.
ALT, alanine aminotransferase; HBV, hepatitis B virus; ULN, upper limit of normal.

Complete response

The proportion of patients who achieved a complete response (HBV-DNA <400 copies/mL by PCR assay and ALT <1.25× ULN and a HBeAg-negative status if they were HBeAg positive at baseline) at Week 48 was similar between groups; 15% (6/40 patients) for the 0.5 mg group and 14% (6/42 patients) for the 1 mg group ($P = 0.855$; Table 2). The durability of responses off treatment in these patients was not assessed, because all patients were enrolled into an entecavir open-label rollover protocol.

Histological response

Paired baseline and Week 48 liver biopsies were available for 26 patients in the 0.5 mg group and 35 patients in the 1 mg group (evaluable biopsy pairs were available in 23 patients in the 0.5 mg group and 35 patients in the 1 mg group). Among patients with evaluable biopsy pairs, histological improvement, defined as a ≥2-point decrease in Knodell HAI and no worsening of fibrosis at Week 48, was seen in 52% (12/23) of patients in the 0.5 mg group and in 60% (21/35) of patients in the 1 mg group (Table 2). Knodell HAI scores were significantly reduced in both treatment groups, dropping by a mean of 3.3 points for the 0.5 mg group and 3.6 points for the 1 mg group ($P < 0.001$ compared with baseline for both groups). Grading of necroinflammation according to the New Inuyama classification was also significantly improved over 48 weeks of treatment in both groups ($P < 0.0001$ compared with baseline for both groups), for 62% (16/26) of patients in the 0.5 mg group and 54% (19/35) of patients in the 1 mg group. No patients exhibited any worsening of grading. There were no significant changes in fibrosis from baseline in either group, as assessed by Knodell fibrosis scores (improvement, no change, and worsening in four, 14, and five patients in the 0.5 mg group and four, 28, and three patients in the 1 mg group, respectively) and staging of New Inuyama classification (improvement, no change, and worsening in seven, 10, and six patients in the 0.5 mg group and six, 26, and three patients in the 1 mg group, respectively).

Resistance analysis

At baseline, rtM204I/V, which is associated with lamivudine resistance, was detected in virus samples from all patients. During the treatment period, only one patient, in the 1 mg group, exhibited increases in HBV-DNA level by 1 $\log_{10}$ copies/mL or more from the nadir (virological breakthrough). There was no emergence of novel amino acid substitutions conferring resistance to entecavir in this patient through to week 48.

A sensitive PCR-based method was used to examine the evolution of amino acid substitutions associated with lamivudine resistance during entecavir treatment in all patients. At week 48, results were available for 39 patients in the 0.5 mg group and 42 patients in the 1 mg group. Substitutions that reverted the YMDD mutants back to wild-type (rtI204M or rtM/I204M) were observed in three of 42 patients in the 1 mg group, whereas no patient in the 0.5 mg group reverted to wild-type at week 48.

Safety

The frequencies of adverse events were similar between treatment groups (Table 3). The majority of adverse events were mild or moderate (grade 1–2) and resolved on treatment in 94% of patients. The most frequent adverse events in both groups were nasopharyngitis, headache, upper respiratory tract infection, nausea, and malaise (Table 3). Serious adverse events included cholecystitis in the 0.5 mg group, and colon polyp, meniscus impairment, and elevation of serum ALT levels in the 1 mg group. In all cases, patients recovered without discontinuation of study