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Extending Combination Therapy with Peginterferon plus Ribavirin for Genotype 2 Chronic Hepatitis C Virological Responders: A Pilot Study of 7 Cases

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Key Words

Hepatitis C virus · Genotype 2 · Interferon · Ribavirin · Combination therapy, extended · Early virological response

Abstract

Objective: In treatment-resistant patients with genotype 2 chronic hepatitis C the suitable treatment duration is still unclear. The aims were to investigate extending combination therapy with peginterferon plus ribavirin for genotype 2. ***Methods:*** 7 patients infected with genotype 2 at a high viral load and who did not achieve a sustained virological response (SVR) with the first course of 24-week IFN plus ribavirin were recruited into the study protocol with a total of 48 weeks of peginterferon plus ribavirin therapy. ***Results:*** SVR was achieved in 5 of 7 patients (71%). All 4 patients (100%) who were in relapse with the first course achieved SVR. Only 1 of 3 patients (33%) who had a non-virological response (NVR) with the first course achieved SVR. All 4 patients who had an early virological response (EVR) with the first course achieved EVR and SVR. Two of 3 patients who had no EVR with the first course also did not achieve EVR and SVR. One patient who had no EVR or a NVR during the first course achieved EVR and SVR with the second course. ***Conclusions:*** Our results suggest that extending combination therapy for genotype 2 chronic hepatitis C might be useful for patients who relapse following 24-week combination therapy.

Introduction

The response to interferon (IFN)-related therapy varies according to hepatitis C virus (HCV) genotype [1, 2]. In Japan, about 70% of patients with chronic hepatitis C are infected with HCV genotype 1b, and about 25% are genotype 2a [3]. The sustained virological response (SVR) to 48-week IFN plus ribavirin combination therapy is about 50% in genotype 1b infection, and the SVR to 24-week combination therapy is more than 80% in genotype 2 infection [4–9].

IFN plus ribavirin combination therapy carries potential serious side effects and is costly especially when used long enough to achieve a high SVR. For these reasons,

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especially in genotype 2 infection, it is necessary to identify those patients who could achieve SVR with a shorter treatment course (16 weeks or less) to free them of unnecessary side effects and reduce costs, preferably as early as possible [6–8]. However, we also sometimes encounter treatment-resistant patients infected with genotype 2 [3, 10, 11]. Our recent report based on 24-week combination therapy showed that 17.5% of patients infected with genotype 2a were not able to achieve SVR, and especially that 81.5 and 18.5% of the non-SVR patients were in relapse or had a non-viral response (NVR), respectively [11]. Thus, the suitable treatment duration, based on the consideration of risk/benefit and cost/benefit, is still unclear in patients infected with genotype 2.

The present study included 7 Japanese adults with genotype 2 and a high viral load, who received a second course of combination therapy. The aims of the study were to investigate extending combination therapy with peginterferon (PEG)-α-2b plus ribavirin for genotype 2 chronic hepatitis C.

Materials and Methods

Study Population

A total of 292 HCV genotype 2-infected Japanese adult patients were consecutively recruited into the study protocol of the combination therapy with IFN (PEG-IFNα-2b or IFNα-2b) plus ribavirin for 24 weeks between March 2002 and September 2008 at Toranomon Hospital, Tokyo, Japan. Among these, 7 of 52 patients who were not able to achieve a sustained virological response were recruited into the study protocol of 48-week combination therapy with PEG-IFNα-2b plus ribavirin. They fulfilled the following inclusion criteria: (1) no SVR with the first course of combination therapy regardless of completing the 24-week therapy; (2) combination therapy was stopped before completing the 24-week therapy due to a decrease in HCV RNA of <2.0 log at 12 weeks after starting treatment based on qualitative PCR analysis [12, 13]; (3) negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp, Emerville, Calif., USA), and positive for HCV RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Pleasanton, Calif., USA); (4) infected with HCV genotype 2a or 2b alone; (5) high viral load (≥100 KIU/ml) by quantitative analysis of HCV RNA with PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems Inc.) within the 2 months preceding enrolment; (6) no hepatocellular carcinoma; (7) body weight >40 kg; (8) no co-infection with human immunodeficiency virus; (9) no treatment with antiviral or immunosuppressive agents within the 3 months preceding enrolment; (10) no alcoholics, lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake); (11) no other form of hepatitis, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease; (12) no pregnant or lactating females; (13) all patients completed a 24-week follow-up program after cessation of treatment and SVR could be evaluated, and (14) each signed a form consenting to the study protocol that had been approved by the human ethics review committee.

Treatment efficacy was defined as: SVR = HCV-RNA-negative based on qualitative PCR analysis 24 weeks after the completion of treatment; relapse = HCV-RNA-negative at completion of treatment but HCV-RNA-positive 24 weeks after the completion, and NVR = HCV-RNA-positive at completion of treatment. Furthermore, an early virological response (EVR) was defined as patients who achieved a decrease in HCV-RNA of >2.0 log within 12 weeks after starting treatment, based on quantitative PCR analysis.

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase and HCV-RNA levels. The serum samples were frozen at −80° within 4 h of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of NS5 region [14]. HCV-RNA levels were measured by quantitative PCR (Amplicor GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems Inc.) at least once every month before, during, and after therapy. The dynamic range of the assay was 5–5,000 KIU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA (<5 KIU/ml) were also checked by qualitative PCR (Amplicor HCV v2.0, Roche Molecular Systems Inc.), which has a higher sensitivity than quantitative analysis, and the results are expressed as positive or negative. The lower limit of the assay was 50 IU/ml.

Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens for examinations contained 6 or more portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histological assessment according to the scoring system of Desmet et al. [15].

Results

Table 1 summarizes the characteristics of the 7 patients at commencement of the second-course combination therapy with PEG-IFN plus ribavirin. There were 5 men and 2 women, aged 40–65 (median 55) years. Two cases were genotype 2a, and the other 5 cases were genotype 2b. They received PEG-IFNα-2b at a median dose of 1.4 (range 1.1–1.7) μg/kg subcutaneously each week. They also received oral ribavirin at a median dose of 10.6

Table 1. Baseline characteristics of patients infected with HCV genotype 2 at the commencement of the second-course combination therapy with peginterferon plus ribavirin, and treatment efficacy of the first and second course of combination therapy

Case No.	Genotype	Sex	Age years	Fibrosis	ALT IU/l	HCV RNA KIU/ml	1st EVR	1st Tx	2nd EVR	2nd Tx
1	2b	M	48	F1	41	5,000	+	relapse	+	SVR
2	2b	F	65	F1	35	1,200	+	relapse	+	SVR
3	2b	M	51	F3	71	310	+	relapse	+	SVR
4	2b	M	56	F1	78	720	+	relapse	+	SVR
5	2a	M	57	F1	240	1,500	–	NVR	+	SVR
6	2a	M	40	F2	434	650	–	NVR	–	NVR
7	2b	F	55	F3	132	1,300	–	NVR	–	NVR

EVR = Early virological response; NVR = non-virological response; SVR = sustained virological response; 1st EVR = EVR with the first course of combination therapy; 2nd EVR = EVR with the second course of combination therapy; Tx = treatment.

(range 7.0–12.6) mg/kg daily. In 3 patients (cases 1, 3, 7), the dose of ribavirin was reduced during treatment due to a fall in Hb concentration. Five patients (cases 1–5) achieved EVR and completed a total of 48 weeks. The other 2 patients did not achieve EVR, so they stopped combination therapy before completing the 48-week therapy (12 weeks for case 6, and 22 weeks for case 7).

Virological Response Rates with the Second Course of Combination Therapy

SVR was achieved by 5 of 7 patients (71.4%). All 4 patients (100%) who were in relapse with the first course of combination treatment achieved SVR with the second course. However, only 1 of 3 patients (33.3%) who had a NVR with the first course achieved SVR. All 4 patients (100%) who had an EVR with the first course achieved EVR and SVR with the second course. However, 2 of 3 patients (cases 6, 7) who had no EVR with the first course also did not have EVR and SVR with the second course. Thus, 2 patients (cases 6, 7) had no EVR and NVR with both the first and second courses, and could not achieve SVR. Interestingly, 1 patient (case 5) who had no EVR or NVR with the first course achieved EVR and SVR with the second course.

Discussion

In patients infected with genotype 1, previous studies have demonstrated that SVR rates of late virological responders (HCV-RNA-positive at 12 weeks and negative 24 weeks after the start of treatment) could be improved when treatment was extended to 72 weeks, compared with a standard treatment duration of 48 weeks, largely as a result of reducing post-treatment relapse rates [16–20]. Thus, prolongation of therapy in genotype 1 may improve the virological response rate. However, it is not clear at present whether prolongation of treatment improves the SVR rate of treatment-resistant Japanese patients infected with genotype 2. This study of patients infected with genotype 2 showed that SVR rates of patients who were EVR and relapsed following the first course with a standard treatment duration of 24 weeks could be improved when treatment was extended to 48 weeks. Interestingly, 1 patient (case 5) who did not have EVR or NVR with the first course achieved EVR and SVR with the second course. This indicates that the SVR rates of patients who had an EVR with the second course might improve further by extending combination therapy regardless of NVR with the first course. To our knowledge, this is the first report to indicate that extending combination therapy to 48 weeks for genotype 2 might be useful.

In this study, 2 patients did not have an EVR or an NVR with both the first and second course and could not achieve SVR. The underlying mechanism(s) of the different virological responses to treatment in patients infected with genotype 2 is still unclear. Previous reports indicated that viral factors (e.g. viral load, aa substitutions in the NS5A region and core region, early viral kinetics, and periods from the start of treatment to initial point of undetectable HCV-RNA) and host factors (e.g. body mass index, fibrosis stage, and hepatocyte steatosis) might be important predictors of treatment response to IFN-related therapy in patients infected with HCV genotype 2a, in addition to treatment-related factors (e.g. treatment duration, and ribavirin dose) [6–11, 21–27]. One of the lim-

itations to this study is that due to the small number of patients we were not able to investigate treatment-resistant factors. Further studies should be performed to identify these viral and host factors before the start of combination therapy. Furthermore, more effective therapeutic regimens, including triple therapy with PEG-IFN plus ribavirin and telaprevir, should be developed for these patients who could not achieve SVR by extending dual therapy of PEG-IFN plus ribavirin.

In conclusion, our results suggest that extending combination therapy to 48 weeks for genotype 2 chronic hepatitis C might be useful for patients who had a relapse following the first course of 24-week combination therapy. In the future a large-scale prospective study based on intention-to-treat analysis should be conducted to confirm the above findings.

Acknowledgement

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Efficacy and Safety of Combination Therapy of Natural Human Interferon β and Ribavirin in Chronic Hepatitis C Patients with Genotype 2 and High Virus Load

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Abstract

Objective The aim of this study was to evaluate the efficacy of combination therapy of natural human interferon-beta and ribavirin in patients infected with hepatitis C virus (HCV) genotype 2 and high virus load.

Methods Inclusion criteria were HCV-genotype 2, serum HCV RNA level of ≥100 KIU/mL before combination therapy. A total of 24 were enrolled in this retrospective cohort study. The treatment period of combination therapy was 24 weeks.

Results Of the 24 study patients, no patient stopped the treatment due to treatment-related adverse events. The dose of drugs were reduced in 8 patients. Twenty-one of 24 patients (87.5%) had sustained virological response (SVR) by the intention to treat analysis. The rate of negative HCV RNA at 8 week after the initiation of treatment was 18/21 (86%) in patients with SVR and 1/3 (33%) in patients with non-SVR. Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 week after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval=1.75-914.78; p=0.021)

Conclusion The combination therapy of IFN-beta and ribavirin offers sufficient safety and efficacy in chronic hepatitis C patients with genotype 2 and high virus load.

Key words: chronic hepatitis C, natural interferon-beta, ribavirin, HCV genotype 2

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Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-10). SVR in the patients with HCV genotype 2 treated with IFN monotherapy for 24 weeks was about 80% in group of low virus load and about 40-45% in high virus load (11). However, it has been reported that the SVR rate was about 80-90% in patients with genotype 2 and high virus load treated with peginterferon and ribavirin for 24 week (12-14). Hence, IFN-monotherapy has been recommended as a first choice for chronic hepatitis C patients with genotype 2 and low virus-load in Japan. On the other hand, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C patients with genotype 2 and high virus-load. Thus, in the present study, we assessed the efficacy of the patients with genotype 2 and high virus load who showed low rate of SVR.

However, the dropout rates in patients treated with combination therapy of peginterferon and ribavirin are higher than those treated with IFN monotherapy (15-17). In particular,

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the adverse events due to combination therapy of IFN and ribavirin have a tendency to occur in elderly patients. Therefore, in the case of elderly patients, the physician in charge often avoids combination therapy of IFN and ribavirin due to side effects. However, recently, the life-span has been long in Japan. Thus, there is an ongoing need to refine treatment strategies with a strong effect and safety in HCV patients.

Festi et al reported that IFN-beta has sufficient tolerability (15). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with a high virus load (11). Enomoto et al have reported that IFN-beta plus ribavirin therapy might seem to have a strong effect and mild side effects originating from treatment (18, 19). However, to date there is little information regarding IFN-beta plus ribavirin therapy for chronic hepatitis C.

Thus, in the present study, we performed a retrospective study to examine the efficacy of combination therapy of IFN-beta and ribavirin in patients with genotype 2 and high virus load.

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 2a or 2b, 2) serum level of HCV RNA of ≥100 KIU/mL before combination therapy; 3) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 4) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 5) leukocytes >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/mL; 6) follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy as well as the potential adverse reactions and informed consent was obtained from each patient.

From December 2004 to May 2008, 24 HCV patients were enrolled in this retrospective cohort study at the study hospital.

A SVR was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver. 2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (20).

Next, predictors of SVR in patients with undetectable HCV RNA in serum during treatment were assessed. Finally, SVR rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy were examined.

Combination therapy of IFN-beta and ribavirin

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital and a signed consent form was obtained each patient. Treatment was provided for 24 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) daily for 2-8 weeks initially, followed by three times a week for 16-22 weeks. Ribavirin (Rebetol, Schering-Plough, Osaka, Japan) were given at the dose described based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for ≤60 kg, 800 mg for >60 kg and ≤80 kg, and 1000 mg for >80 kg). The period of daily administration in IFN-beta treatment was determined by the physician. The patients were divided into three groups based on the difference of period of daily administration of IFN-beta at the initial stage of treatment: a 2-week regimen, 10 patients; a 4-week regimen, 5 patients; and an 8-week regimen, 9 patients.

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80℃ until analyzed. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (21). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (22). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (20). Clinical evaluation and biochemical and hematological tests were performed at 4 weekly intervals.

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test, Fisher' exact test, Kruskal Wallis test, and/or logistic regression analysis. The following variables were evaluated as prognostic factors: sex, age, body mass index, a history of interferon therapy, a HCV RNA level, biochemical factors (AST, ALT, triglyceride, HDL-cholesterol, LDL-cholesterol), platelet count, HCV RNA 4, 8, 12 weeks after the initiation of IFN therapy, continuous negative period of HCV RNA during IFN therapy and period of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Result

Clinical characteristics of the patients

A total of 24 patients were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age=55.9 years, male/female=11/13, and median

Table 1. **Clinical Backgrounds before Combination Therapy of Peginterferon and Ribavirin in Chronic Hepatitis C Patients**

Character	value
Patients, n	24
Sex, male (%)	11(45.8%)
Age (yrs)	55.9±10.2
BMI	23.0±2.5
A history of IFN (+)	12 (50.0%)
HCV RNA(KIU/mL)	870 (43-5000)
HCV genotype (2a·2b)	14/10
AST (IU/L)	71±51
ALT (IU/L)	130±122
FPG (mg/dl)	96±13
Triglyceride (mg/dl)	111±73
HDL cholesterol (mg/dL)	52±19
LDL cholesterol (mg/dL)	117±31
Platelet (10^4/mm^3)	16.6±4.5
A regimen of daily administration of IFN-beta* (2-week·4-week·8-week)	10/5/9

Data are number of patients (percentage) or mean ± standard deviation

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon;

*The patients were divided into three groups based on the difference of period of daily administration of IFN-beta at the initial stage of treatment: a 2-week regimen of daily administration of IFN-beta, 10 patients; a 4-week regimen, 5 patients; and an 8-week regimen, 9 patients.

(range) HCV-RNA=870(103-5,000) KIU/mL.

Safety and tolerance of IFN

Of the 24 patients included in this study, none of the patients discontinued combination therapy because of IFN-related adverse events. However, 7 out of 24 patients had dose reduction of interferon and/or ribavirin due to side effects. IFN-beta dose reduction was necessary in one case due to the development of neutropenia. RBV dose reduction was applied in 6 patients, due to anemia.

The leukocyte count was 4,700 ± 1,390/mm^3 and the platelet count was 166,000 ± 45,000/mm^3 before the initiation of IFN therapy, whereas the values were 3,020 ± 1,05/mm^3 and 134,000 ± 39,000/mm^3, respectively, two weeks after the initiation of the therapy.

Efficacy of treatment

Out of the 24 patients enrolled in the present study, 21 patients (87.5%) had SVR by the intention-to-treat analysis. Patients aged ≥65 years were five in total. Four out of five patients aged ≥65 years had SVR. Table 2 shows the differences in the clinical background between patients with SVR and those without SVR. The rate of negative HCV RNA at 8 weeks after the initiation of treatment was 18/21(86%) in patients with SVR and 1/3 (33%) in patients with non-SVR. Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 weeks after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval=1.75-914.78; P=0.021). Moreover, the SVR was not significantly different based on the difference of period of daily administration of IFN-beta at the initial stage of treatment.

Background of non-SVR cases

Three patients had negative HCV RNA at the end stage of treatment, but showed reappearance of HCV RNA after

Table 2. **The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR**

	SVR (n=21)	Non-SVR (n=3)	p value*
Age (years old)	56.1 ± 9.1	57.0 ± 8.0	0.827
Sex (male/female)	12/9	2/1	0.449
BMI	22.9 ± 2.5	22.8 ± 2.6	1.000
a history of IFN (+/-)	11/10	1/2	0.759
HCV-load (KIU/mL)	794± 786	1545± 1797	0.759
AST (IU/L)	69 ± 47	44 ± 12	0.540
ALT (IU/L)	83 ± 39	70 ± 55	0.359
FPG (mg/dL)	96 ± 13	92 ± 3	0.813
Triglyceride (mg/dL)	112 ± 74	107 ± 57	0.614
HDL cholesterol (mg/dL)	51 ± 20	65 ± 17	0.297
LDL cholesterol (mg/dL)	113 ± 31	126 ± 15	0.540
Platelet (10^4/mm^3)	16.3 ± 4.7	17.7 ± 5.3	0.701
HCV RNA (+/-) 4W	9/12	2/1	0.576
HCV RNA (+/-) 8W	3/18	2/1	0.099, 0.021
HCV RNA (+/-) 12W	0/21	0/3	1.000
Period of daily administration of IFN* (2-week/4-week/8-week)	9/4/8	1/1/1	0.925

Data are number of patients (percentage) or mean±standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon;

*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 2-8 weeks, followed by three times a week for 16-22 weeks. Figure of 2, 4, and 8 represents the (week) of daily administration of IFN-beta at the initial stage.

†Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test, Fisher' exact test, Kruskal wallis test.

‡Logistic regression analysis showed that SVR occurred when serum HCV RNA at 8 week after the initiation of combination therapy was negative (hazard ratio: 40.0; 95% confidence interval =1.75-914.78; p = .021)

the termination of treatment. Clinical backgrounds of these three cases with relapse of HCV RNA after the termination of treatment are shown in Table 3. In case 1 and 2, the attainment time of negativity of serum HCV RNA was 12 weeks after the initiation of treatment. In case 3, the adherence of both drugs of IFN-beta and ribavirin was less than two-third compared to scheduled dose.

Discussion

We have described the efficacy of combination therapy of IFN-beta and ribavirin in patients infected with HCV genotype 2a or 2b. The present study was limited to small size with genotype 2 and HCV-load of ≥100 KIU/mL and high virus load before combination therapy. SVR in the patients with genotype 2 treated with IFN monotherapy for 24 weeks was about 80% in the group with a low virus load and about 40-45% with high virus load (11). Thus, in the present study, we assessed the efficacy of the patients with genotype 2 and a high virus load who showed low rate of SVR. Moreover, 7 of 24 patients did not have a histological examination of the liver within one year before combination

Table 3. **Clinical Backgrounds of Patients with Non-SVR**

Case	Age/Sex	genotype	HCV RNA	AST/ALT (IU/L)	response*	Adherence (%)	
						IFN	RBV
1	53/M	2a	220	51/104	12W	104%	100%
2	67/M	2b	5000	30/27	12W	82%	84%
3	51/F	2a	103	50/51	4W	62%	68%

Data are number of patients (percentage) or mean±standard deviation.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon; RBV, ribavirin

*Response of HCV RNA means attainment time of negativity of serum HCV RNA after the initiation of combination therapy

therapy. Another limitation is that the present study was not a randomized controlled study.

However, several findings from the present study have direct implications for combination therapy for chronic hepatitis C in the future. First, the present results suggest that drop-out rate due to side effects in combination therapy of IFN-beta and ribavirin is low. In the previous study, we have reported that the drop out rate due to side effects in combination study of peginterferon and ribavirin was 8.4% in 0.5 year after the initiation of treatment and 14.9% in one year (15). In the present study, none of the patients discontinued combination therapy because of IFN-related adverse events.

Secondly, out of 24 patients given the combination therapy, 21 patients had SVR. This SVR rate is similar to that of the 24-week combination therapy of peginterferon and ribavirin reported previously (11-13).

Third, the patients with genotype 2 have the possibility of non-SVR in a regimen for 24-weeks when the attainment time of negativity of serum HCV RNA is longer than 8 weeks after the initiation of combination therapy. This indicates that patients with delayed undetectable HCV RNA should be treated to continue the negativity of serum HCV RNA for a prolonged period of >24 weeks to obtain a high rate of SVR.

IFN-beta should be given intravenously. The intravenous injection is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to combination therapy of IFN alpha and ribavirin (18, 19). Moreover, IFN beta induced mental disorders are milder than those induced by IFN alpha (23). Thus, IFN beta could be given in elderly patients of ≧65 years because of mild side effects (24).

In conclusion, the combination therapy of IFN-beta and ribavirin offers sufficient safety and efficacy in chronic hepatitis C patients with genotype 2 and a high virus load.

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HEPATOLOGY

Efficacy of switching to entecavir monotherapy in Japanese lamivudine-pretreated patients

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Abstract

Background and Aims: To assess the efficacy of switching Japanese chronic hepatitis B patients from lamivudine monotherapy to entecavir 0.5 mg/day.

Methods: A retrospective analysis was conducted on 134 patients switched to entecavir between September 2006 and February 2008 for 6 months or more. Patients were divided into three groups based on viral load at entecavir switching point (baseline < 2.6, 2.6–5.0 and > 5.0 $\log_{10}$ copies/mL).

Results: At baseline, detection of lamivudine-resistant virus was highest in patients with higher hepatitis B virus (HBV) DNA (76% *vs* 23% in ≥ 2.6 and < 2.6 $\log_{10}$ copies/mL, respectively), and in patients with longest previous exposure to lamivudine (52%, 28% and 24% for > 3 years, 1–3 years and < 1 year, respectively). Two years after entecavir switching, HBV DNA suppression to less than 2.6 $\log_{10}$ copies/mL was achieved in 100% (32/32), 92% (12/13) and 44% (4/9) of patients in the less than 2.6, 2.6–5.0 and more than 5.0 $\log_{10}$ copies/mL baseline groups, respectively. Alanine aminotransferase (ALT) normalization occurred in 76–96% and 90–100% of patients following 1 and 2 years of entecavir treatment, respectively. One patient (2.6–5.0 $\log_{10}$ copies/mL) with lamivudine-resistant mutants at baseline developed entecavir resistance at week 48 during follow up.

Conclusion: Switching to entecavir 0.5 mg/day achieves or maintains undetectable HBV DNA levels and ALT normalization over 2 years, especially in patients with viral load less than 5.0 $\log_{10}$ copies/mL.

Introduction

Hepatitis B virus (HBV) infection is a serious public health threat affecting 350–400 million people worldwide, the majority of whom live in the Asia–Pacific region.[1,2] Chronically-infected people are at risk of developing cirrhosis, liver failure and hepatocellular carcinoma. Studies have suggested that high serum HBV DNA is a key risk predictor of chronic hepatitis B (CHB) complications.[3,4] Therefore, the main purpose of CHB therapies is to permanently suppress viral replication and sustain viral suppression to prevent long-term liver damage.[2,5,6]

Lamivudine was the first nucleoside analog to be widely prescribed for CHB patients, mainly due to its antiviral efficacy and safety profile.[2] However, lamivudine's long-term efficacy is diminished by the emergence of drug-resistant substitutions, generally in the tyrosine–methionine–aspartate–aspartate (YMDD) motif of the reverse transcriptase (rt) polymerase gene.[7–9] Detection of lamivudine-resistant HBV substitutions occurs in 15–30% and 70% of patients after 1 and 5 years of treatment, respectively.[8] Continuing lamivudine monotherapy in the presence of lamivudine resistance is not recommended because it is no longer effective in suppressing viral replication.[2] Furthermore, the initial improvement in histology and clinical benefits may be reversed or decreased due to the emergence of lamivudine-resistant substitutions.

Antiviral efficacy of entecavir (0.5 mg/day) as first-line therapy was superior to lamivudine in treatment-naïve patients on all virological, biochemical and histological end-points after 48 weeks of treatment,[10–14] with very low rates of emergence of viral resistance (1.2% after 5 years of entecavir treatment).[15,16] Entecavir has a high genetic barrier to resistance,[17–19] requiring multiple substitutions (including YMDD mutations) to express viral resistance.[16–21] In agreement with this, entecavir-resistant mutants emerge more frequently in lamivudine-refractory patients.[22,23] In a study of hepatitis B e antigen (HBeAg)-positive lamivudine-refractory patients with high HBV DNA levels at baseline (mean > 9 $\log_{10}$ copies/mL), switching to entecavir 1 mg/day achieved HBV DNA suppression to undetectable levels (< 300 copies/mL; 40%, 96 weeks) and alanine aminotransferase (ALT) normalization (81%, 96 weeks) at higher proportions than continued lamivudine

monotherapy,[22] although response to therapy was less pronounced than in treatment-naïve patients with comparable baseline levels of HBV DNA.[10,13,14] The probability of achieving HBV DNA suppression to undetectable levels at 96 weeks with entecavir was 73% in patients whose baseline HBV DNA was less than 7 $\log_{10}$ copies/mL ($n = 11$), and none of these patients developed entecavir resistance.[22]

In a randomized controlled trial of lamivudine-refractory Japanese patients with mean HBV DNA at baseline of 7.6–7.7 $\log_{10}$ copies/mL, switching to entecavir (0.5 or 1 mg/day) for 48 weeks achieved HBV DNA suppression to below detectable levels in 33% of patients in the entecavir dose groups, and ALT normalization in 78–86%.[24] Switching to entecavir in patients with evidence of lamivudine-resistant substitutions and low viral load at switching point has not been prospectively investigated in Japanese patients. There are limited data concerning the efficacy of entecavir in lamivudine-pretreated patients who have not developed lamivudine resistance.

The objective of this study was to assess the efficacy of switching to entecavir 0.5 mg/day in Japanese lamivudine-pretreated patients whose HBV DNA levels at switching point (baseline) ranged from less than 2.6 to 7.6 $\log_{10}$ copies/mL, with or without lamivudine-resistant substitutions.

Methods

Design and setting

A retrospective analysis of a CHB patient population ($n = 134$) at Toranomon Hospital (Tokyo, Japan) was performed to identify patients switched from lamivudine 100 mg/day monotherapy to entecavir 0.5 mg/day between September 2006 and February 2008, and who had received entecavir for at least 6 months. Among all patients selected, only one had a history of adefovir add-on therapy prior to switching to entecavir (case report). Conserved serum from all patients was analyzed to determine baseline characteristics and study end-points.

Study end-points

Clinical efficacy of entecavir was assessed as the proportion of patients achieving HBV DNA suppression to undetectable levels (< 400 copies/mL or < 2.6 $\log_{10}$ copies/mL), and patients achieving ALT normalization (normal ALT levels: men 8–42 IU/L, women 6–27 IU/L). HBV DNA was measured using the polymerase chain reaction (PCR)-based Amplicor HBV Monitor assay (Roche Diagnostics, Indianapolis, IN, USA; lower limit of detection of < 2.6 $\log_{10}$ copies/mL).[25] HBeAg loss in patients who were HBeAg-positive at baseline was also analyzed. Measurements were made from conserved samples taken at baseline, and after 6 months, 1 and 2 years from entecavir treatment initiation.

Assessment of viral resistance

Conserved serum was used to detect the presence of viral lamivudine-resistant rtM204V/I substitutions in all patients at baseline, and following the entecavir switch in patients treated with entecavir for at least 6 months. Lamivudine-resistant virus (rtM204V/I or YMDD motif substitutions) was analyzed using a combination of the quantitative enzyme-linked immunosorbent assay standardized using a purified *Taenia solium* cysticerci fraction (PCR enzyme-linked immunosorbent assay) and the enriched PCR enzyme linked minisequence assay.[26] Direct sequencing of HBV DNA polymerase reverse transcriptase site was also performed.[27] Detection of entecavir-resistant virus was conducted using direct sequencing of HBV DNA polymerase reverse transcriptase site.[27]

Data analyses

Statistical comparisons between treatment groups were assessed using χ^2-test and Kruskal–Wallis test where appropriate. Calculations were performed using StatView software (ver. 4.5J; Abacus Concepts, Berkeley, CA, USA). A two-tailed P-value less than 0.05 was considered statistically significant.

To identify predictive factors of HBV DNA negativity (suppression to below detectable levels) after 6 months of the entecavir switch, univariate and multivariate logistic regression analyses were carried out. Potential predictive factors at baseline included: sex; age; levels of aspartate aminotransferase (AST), ALT, albumin, γ-glutamyl transpeptidase, total bilirubin and α-fetoprotein; platelet count; viral load; liver disease stage (cirrhosis or other); family history; HBV genotype; lamivudine treatment duration prior to entecavir switch; HBeAg status; and lamivudine resistance. Each variable was transformed into categorical data consisting of two simple ordinal numbers. All factors that were at least marginally associated with HBV DNA negativity ($P < 0.10$) were used in a multiple logistic regression analysis. To assess relative risk confidence, odds ratio (OR) and 95% confidence interval (CI) were calculated. All analyses were performed using SPSS II software ver. 11.0 (SPSS, Chicago, IL, USA).

Results

Patient characteristics before switching to entecavir

Lamivudine-pretreated patients switched to entecavir 0.5 mg/day ($n = 134$) were divided into three groups based on their HBV DNA level at the switching point: HBV DNA of less than 2.6 $\log_{10}$ copies/mL ($n = 92$), 2.6–5.0 $\log_{10}$ copies/mL ($n = 25$) and more than 5.0 $\log_{10}$ copies/mL ($n = 17$) (Table 1). Patients with HBV DNA levels of more than 5.0 $\log_{10}$ copies/mL had the highest AST/ALT levels and highest proportion of HBeAg-positive cases ($P < 0.05$). These patients had been treated with lamivudine for the shortest time period compared to patients from the two other groups ($P < 0.05$; Table 1).

Viral resistance to lamivudine at baseline

At baseline, lamivudine-resistant rtM204V/I mutant virus was detected in 23% of patients with HBV DNA of less than 2.6 $\log_{10}$ copies/mL, compared to 76% in each of the HBV DNA 2.6–5.0 $\log_{10}$ copies/mL and more than 5.0 $\log_{10}$ copies/mL groups (Table 2). In all treatment groups, a higher occurrence of resistant virus was observed with longer exposure to lamivudine, independent of viral DNA levels.

Table 1 Patient characteristics at point of switching to entecavir (baseline) and entecavir treatment duration

	All patients	Serum HBV DNA levels by baseline treatment group, log_{10} copies/mL			
		< 2.6	2.6–5.0	> 5.0	*P**
Patients, *n*	134	92	25	17	
Sex, *n* male/female	94/40	67/25	19/6	8/9	0.08
Age, years†	53 (23–83)	53 (27–83)	50 (32–77)	37 (23–77)	0.036
Bilirubin, mg/dL†	0.6 (0.2–3.4)	0.6 (0.2–3.4)	0.6 (0.3–1.8)	0.7 (0.3–1.2)	0.53
AST, IU/L†	24 (13–451)	23 (13–53)	23 (14–50)	37 (14–451)	0.0083
ALT, IU/L†	21 (8–1382)	21 (8–56)	20 (10–111)	46 (9–1382)	0.0002
Albumin, g/dL†	3.9 (2.7–4.8)	3.9 (2.7–4.4)	4.0 (3.3–4.8)	3.9 (3.6–4.6)	0.94
Histology, *n* CH/LC	89/45	56/36	19/6	14/3	0.11
HBeAg, *n* ±	30/104	11/81	5/20	14/3	< 0.0001
HBV DNA, log_{10} copies/mL†	< 2.6 (< 2.6–7.6)	< 2.6	3.9 (2.7–5.0)	6.5 (5.1–7.6)	–
Genotype, *n* A/B/C/unknown	3/9/115/7	2/6/78/6	1/2/22/0	0/1/15/1	0.87
Treatment duration, months†					
Lamivudine	36 (0.5–103)	36 (3–103)	70 (2–89)	17 (0.5–89)	0.009
Entecavir‡	21 (6–33)	20 (6–33)	24 (6–32)	27 (6–33)	0.034

*Comparison of the three patient subgroups using the Kruskal–Wallis test; $P < 0.05$ was considered statistically significant.
†Data are median (range).
‡Entecavir treatment duration is from point of switching.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CH, chronic hepatitis; HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; LC, liver cirrhosis.

Table 2 rtM204V/I mutant occurrence at baseline of switching to entecavir

	Duration of previous lamivudine treatment, years			All patients
	< 1	1–3	≥ 3	
Baseline treatment group				
< 2.6 log_{10} copies/mL	1/10 (10%)	4/35 (11%)	16/47 (34%)	23%
2.6–5.0 log_{10} copies/mL	1/5 (20%)	3/4 (75%)	15/16 (94%)	76%
> 5.0 log_{10} copies/mL	3/6 (50%)	6/7 (86%)	4/4 (100%)	76%
All patients	24%	28%	52%	–

Clinical efficacy of entecavir 0.5 mg/day

Switching to entecavir 0.5 mg/day for 1 year resulted in HBV DNA suppression to undetectable levels in the majority of patients with HBV DNA below 5.0 log_{10} copies/mL (100% and 96% for HBV DNA < 2.6 and 2.6–5.0 log_{10} copies/mL, respectively) (Table 3). This proportion was slightly decreased when previous lamivudine treatment duration exceeded 3 years in the 2.6–5.0 log_{10} copies/mL group. In the HBV DNA more than 5.0 log_{10} copies/mL group, approximately half (41%) of the patients achieved viral suppression after 1 year (Table 3); entecavir's efficacy seemed to decrease with prolonged previous exposure to lamivudine, with only 25% of patients having more than 3-year lamivudine treatment achieving undetectable viral load. Similarly, after 2 years, HBV DNA suppression was achieved by 100% and 92% of patients in the HBV DNA less than 2.6 and 2.6–5.0 groups, respectively, and by 44% of patients in the HBV DNA more than 5.0 log_{10} copies/mL group (Table 3).

Among those who failed to suppress viral load, only one case of virological breakthrough was found (2.6–5.0 log_{10} copies/mL group; described under case report). This patient had been previously exposed to lamivudine for more than 3 years.

Alanine aminotransferase levels were normalized in 76–96% and 90–100% of patients following 1 and 2 years of entecavir treatment, respectively (Table 3). HBeAg loss was observed in 27% (3/11), 20% (1/5) and 29% (4/14) of patients with HBV DNA of less than 2.6, 2.6–5.0 and more than 5.0 log_{10} copies/mL, respectively, in the first year.

Lamivudine-resistant substitutions in patients switched to entecavir

Of the 130 patients who received entecavir treatment for at least 1 year, 11 cases failed to suppress HBV DNA to below less than 2.6 log_{10} copies/mL and remained HBV DNA-positive in the first year (1 and 10 in the HBV DNA 2.6–5.0 and > 5.0 log_{10} copies/mL groups, respectively; Table 3). Serum HBV DNA analysis confirmed the presence of rtM204V/I substitutions in 10 of these patients, of which six were rtM204I and three were rtM204V substitutions (Table 4); the remaining patient (2.6–5.0 log_{10} copies/mL group; previous lamivudine exposure 5 years) carried a mixed type substitution, rtM204I plus rtM204V. The only HBV DNA-positive patient who did not

Table 3 Clinical efficacy of entecavir 0.5 mg/day in lamivudine-pretreated patients

End-point by baseline treatment group	Duration of entecavir treatment		
	6 months	1 year	2 years
HBV DNA suppression to undetectable levels, *n*/N (%)			
< 2.6 $\log_{10}$ copies/mL	90/92 (98%)	89/89 (100%)	32/32 (100%)
Previous lamivudine < 1 year	10/10 (100)	9/9 (100)	5/5 (100)
Previous lamivudine 1–3 years	35/35 (100)	35/35 (100)	14/14 (100)
Previous lamivudine > 3 years	45/47 (96)	45/45 (100)	13/13 (100)
2.6–5.0 $\log_{10}$ copies/mL	24/25 (96%)	23/24 (96%)	12/13 (92%)
Previous lamivudine < 1 year	5/5 (100)	5/5 (100)	3/3 (100)
Previous lamivudine 1–3 years	4/4 (100)	4/4 (100)	2/2 (100)
Previous lamivudine > 3 years	15/16 (94)	14/15 (93)	7/8 (88)
> 5.0 $\log_{10}$ copies/mL	5/17 (29%)	7/17 (41%)	4/9 (44%)
Previous lamivudine < 1 year	2/6 (33)	3/6 (50)	2/4 (50)
Previous lamivudine 1–3 years	2/7 (29)	3/7 (43)	2/4 (50)
Previous lamivudine > 3 years	1/4 (25)	1/4 (25)	0/1 (0)
ALT normalization, *n*/*n* (%)			
< 2.6 $\log_{10}$ copies/mL	88/92 (96%)	83/89 (93%)	32/32 (100%)
2.6–5.0 $\log_{10}$ copies/mL	24/25 (96%)	23/24 (96%)	12/13 (92%)
> 5.0 $\log_{10}$ copies/mL	14/17 (82%)	13/17 (76%)	9/10 (90%)

ALT, alanine aminotransferase; HBV, hepatitis B virus.

Table 4 HBV DNA positive rates in patients switched to entecavir 0.5 mg/day for at least 1 year

	HBeAg status	YMDD motif substitution	HBV DNA positive rate, *n*/N (%)	Duration of previous lamivudine treatment, years per patient
Baseline treatment group				
< 2.6 $\log_{10}$ copies/mL	Positive	Wild (or none)	0/10 (0%)	n/a
		YIDD	0/1 (0%)	n/a
	Negative	Wild (or none)	0/58 (0%)	n/a
		YIDD	0/15 (0%)	n/a
		YVDD	0/4 (0%)	n/a
		YIDD + YVDD	0/1 (0%)	n/a
2.6–5.0 $\log_{10}$ copies/mL	Positive	Wild (or none)	0/4 (0%)	
		YIDD + YVDD	1/1 (100%)†	5.0
	Negative	Wild (or none)	0/2 (0%)	n/a
		YIDD	0/10 (0%)	n/a
		YVDD	0/6 (0%)	n/a
		YIDD + YVDD	0/1 (0%)	n/a
> 5.0 $\log_{10}$ copies/mL	Positive	Wild (or none)	1/4 (25%)	0.2
		YIDD	6/9 (67%)	0.5; 1.3; 1.5; 2.7; 3.9; 7.4
		YVDD	1/1 (100%)	0.7
	Negative	YIDD	0/1 (0%)	n/a
		YVDD	2/2 (100%)	1.8; 4.5
All patients			11/130 (8%)	

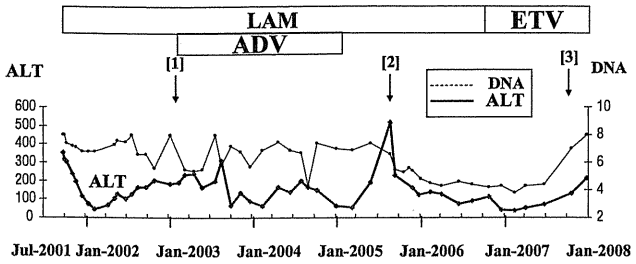
YMDD motif substitutions: wild, rt204M; YIDD, rt204I; YVDD, rt204V; YIDD + YVDD, rt204I + rt204V.
†Patient with lamivudine-resistant HBV who developed entecavir resistance.
HBeAg, hepatitis B early antigen; HBV, hepatitis B virus; n/a, not available.

carry any detectable lamivudine-resistant substitution had the shortest previous lamivudine exposure (< 6 months; Table 4).

Of the 10 patients carrying rtM204V/I substitutions, eight were HBeAg-positive; the other two patients were HBeAg-negative and carried a lamivudine-resistant rtM204V type substitution.

Emergence of entecavir-resistant mutant: case report

One patient (2.6–5.0 $\log_{10}$ copies/mL group) carrying a mixed substitution YIDD + YVDD (rtM204I + rtM204V) developed entecavir resistance with a recognized rtS202G substitution



Amino acid (reverse transcriptase)

	180	202 204	221 223	229
AB014394	SPFLLAQFTSAI	CLAFSYMDDVVLG	FTSVTNFLLS	
[1] February 2003	------------	-------------	----------	
[2] August 2005	----M-------	------V------	----------	
	------------	------I------	----------	
[3] October 2007	----M-------	----G-V------	Y-A-----V-	

Figure 1 Clinical course and evolution of viral polymerase reverse transcriptase gene sequence in a patient with confirmed rtM204V/I substitutions (YIDD + YVDD) and emerging entecavir resistance substitution (rtS202G). AB014394 was a strain reported by Takahashi *et al.*[33] Two kinds of strains emerged in August 2005 (rtL180M/rtM204V and rtM204I). In October 2007, an additional amino acid substitution (rtS202G) was detected. ADV, adefovir; ALT, alanine aminotransferase; ETV, entecavir; LAM, lamivudine. — DNA; — ALT.

(Table 4). Figure 1 describes the clinical course and evolution of viral DNA sequence. This 37-year-old Japanese man was found to be seropositive for hepatitis B surface antigen with mild ALT elevation in December 1998. He was diagnosed with CHB by peritoneoscopy and liver biopsy (mild hepatitis [A1] and mild fibrosis [F1]). HBeAg was positive; serum HBV DNA was more than 7.6 $\log_{10}$ copies/mL. Treatment with lamivudine 100 mg/day was initiated in October 2001, at which time serum HBV DNA was more than 7.6 $\log_{10}$ copies/mL and ALT was 314 IU/L. In February 2003, adefovir dipivoxil 10 mg/day was added-on to lamivudine, but failed to decrease HBV DNA load. In January 2005, adefovir was withdrawn; the patient remained on lamivudine monotherapy. Amino acid substitutions of the rt gene, rtL180M, rtM204V and rtM204I were detected in August 2005. In October 2006, the patient was switched directly from lamivudine to entecavir 0.5 mg/day without treatment interruption. In February 2007, ALT levels decreased to within normal values, and serum HBV DNA was less than 4 $\log_{10}$ copies /mL. However, shortly after, both ALT levels and HBV DNA began to rise again. In October 2007, amino acid substitutions rtL180M, rtM204V and rtS202G were detected.

Predictive factors of HBV DNA negativity

Univariate analyses identified six factors that correlated with HBV DNA suppression to undetectable levels after 6 months of the entecavir switch: viral load less than 5 $\log_{10}$ copies/mL ($P < 0.001$); HBeAg-negative status ($P < 0.001$); the absence of lamivudine resistance ($P < 0.001$); normal AST level (≤ 33 IU/L; $P = 0.008$); normal ALT level (men ≤ 42 IU/L, women ≤ 27 IU/L; $P < 0.001$); and chronic hepatitis stage of liver disease ($P = 0.069$). Multivariate analyses showed that viral load below 5 $\log_{10}$ copies/mL (OR = 69.03; 95% CI = 13.23–360.09; $P < 0.001$) and the absence of lamivudine resistance (OR = 8.17; 95% CI = 1.25–53.34; $P = 0.028$) each independently influenced entecavir's efficacy to suppress HBV DNA to undetectable levels after 6 months.

Discussion

Entecavir is recommended as a first-line CHB treatment by all major guidelines, due to its antiviral potency and high genetic barrier to resistance in nucleos(t)ide-naïve patients.[2,5,6] Conversely, in lamivudine-resistant patients, switching to entecavir is not a first-choice treatment, due to increased risk of emergence of entecavir resistance on a multiple substitution background.[22,23] However, in attempts to rescue those with suboptimal antiviral response and also to avoid the emergence of viral resistance in responsive patients during their treatment course, switching to entecavir is recommended by the Japanese Ministry of Health, Welfare and Labor for lamivudine-pretreated patients with undetectable viral load (< 2.6 $\log_{10}$ copies/mL), and for patients with detectable HBV DNA but without biochemical breakthrough and lamivudine resistance.[28] This study provides a unique opportunity to evaluate the efficacy of entecavir in a lamivudine-pretreated population with low viral load at switching point.

The majority of patients with HBV DNA at baseline of less than 5 $\log_{10}$ copies/mL maintained or achieved viral suppression 1 year after switching to entecavir, despite 23–76% of them carrying lamivudine-resistant substitutions. A similar trend was maintained during the second year. Conversely, viral suppression below detection limits was reported in less than half of patients with high viral load at baseline (HBV DNA 5.1–7.6 $\log_{10}$ copies/mL) carrying rtM204V/I substitutions (76% patients), in agreement with earlier studies showing diminished entecavir efficacy in lamivudine-refractory patients with elevated viral load.[22,23,29] In addition, multivariate analyses revealed that a viral load of less than 5 $\log_{10}$ copies/mL was an independent predictive factor of HBV DNA suppression to undetectable levels, after 6 months of entecavir therapy. Taken together, these data suggest that switching to entecavir is mostly efficacious in patients with low viral load regardless of the presence of rtM204V/I substitutions. This observation adds another perspective in predicting clinical response to entecavir in lamivudine-pretreated patients.

Another predictive factor of entecavir's efficacy in this retrospective cohort is the absence of lamivudine resistance. This is consistent with previous research suggesting decreased genetic barrier of entecavir to resistance in the presence of lamivudine-resistant substitutions.[22,23] The responsiveness of lamivudine-resistant patients with low viral load reported here could be explained by the ability of entecavir to clear low loads of rtM204V/I mutants. This is suggested by *in vitro* data showing maintained sensitivity of lamivudine-resistant mutants to entecavir, although at higher EC_{50}. Assessing the kinetics of rtM204V/I mutants in response to entecavir switching in patients with undetectable viral load is worth further characterization.

Previous studies have shown that developing entecavir resistance is higher in the presence of pre-existing lamivudine-resistant substitutions.[16–21,30] Despite the presence of lamivudine-resistant virus in 23%–76% of all patient groups, the emergence of entecavir resistance was rare, with only one confirmed case from the

HBV DNA 2.6–5.0 $\log_{10}$ copies/mL group. This patient's history is suggestive of a typical refractory case, with failure of multiple regimens including the combination of lamivudine plus adefovir (Fig. 1). The low entecavir resistance rate in this study may be due to the relatively short treatment period and small sample size. Further follow up will be required to monitor for subsequent emergence of entecavir resistance in these patients.

One could argue whether it is cost-effective to switch all lamivudine-treated patients with undetectable HBV DNA to entecavir. The GLOBE study demonstrated that although fewer lamivudine-treated patients with undetectable HBV DNA at week 24 developed viral resistance, resistance could still occur after 2 years of treatment (9% and 5% of HBeAg-positive and HBeAg-negative patients, respectively).[31] Moreover, Yuen and collaborators also reported that of lamivudine-treated patients who achieved HBV DNA suppression below 200 copies/mL at week 24, 8.3% developed resistance after 5 years.[32] In countries where medicine access is an issue, further studies are needed to evaluate the cost-effectiveness of entecavir switching of all patients with undetectable viral load, versus switching only those at risk of developing viral resistance. Comparative studies integrating the efficacy and safety of standard adefovir add-on versus switching to entecavir monotherapy are also warranted in these patients.

Study limitations should be considered. This is a retrospective analysis of CHB patients which, in the absence of matching controls, may introduce confounding errors and bias. Specifically, a control arm for the HBV PCR-negative group (< 2.6 $\log_{10}$ copies/mL; $n = 92$) would be required to strengthen study conclusions. Another limitation is the small sample size of the intermediate and high HBV DNA cohorts (25 patients with 2.6–5.0 $\log_{10}$ copies/mL, and 17 patients with > 5.0 $\log_{10}$ copies/mL, respectively); adding more patients to these samples as available would add weight to describing higher number entecavir response and resistance rates in these groups.

In conclusion, this study shows that the efficacy of switching from lamivudine to entecavir 0.5 mg/day is highest for Japanese patients with no rtM204V/I substitutions and a viral load of less than 5 $\log_{10}$ copies/mL, independent of their previous exposure to lamivudine. Efficacy is decreased for patients with rtM204V/I substitutions and low viral load, and is lowest for patients with rtM204V/I substitutions and high viral load. Viral resistance to entecavir after 48 weeks is rare in these patients. Multivariate analyses showed that viral load of less than 5 $\log_{10}$ copies/mL and the absence of lamivudine resistance are independent factors predicting entecavir's efficacy to reduce HBV DNA to undetectable levels after 6 months of treatment.

Acknowledgments

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CLINICAL STUDIES

HBcrAg is a predictor of post-treatment recurrence of hepatocellular carcinoma during antiviral therapy

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Keywords
covalently closed circular DNA – HBcrAg – HCC recurrence – nucleot(s)ide analogue – portal vein invasion

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Abstract

Background/Aims: The recurrence rate of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) is high even in patients receiving curative therapy. In this study, we analysed the risk factors for tumour recurrence after curative therapy for HBV-related HCC while under treatment with nucleot(s)ide analogues (NAs) by measuring serum HBcrAg and intrahepatic covalently closed circular DNA (cccDNA) levels to elucidate the viral status associated with HCC recurrence. *Methods:* We enrolled 55 patients who developed HCC during NA therapy and underwent either curative resection or percutaneous ablation for HCC. *Results:* Hepatocellular carcinoma recurred in 21 (38%) of the patients over a period of 2.2 (range, 0.2–7.4) years. In multivariate analysis, serum HBcrAg levels $\geq$4.8log U/ml at the time of HCC diagnosis (hazard ratio, 8.96; 95% confidential interval, 1.94–41.4) and portal vein invasion (3.94, 1.25–12.4) were independent factors for HCC recurrence. The recurrence-free survival rates of the high cccDNA group were significantly lower than those of the low cccDNA group only in patients who underwent resection ($P = 0.0438$). A positive correlation ($P = 0.028$; $r = 0.479$) was observed between the intrahepatic cccDNA and the serum HBcrAg levels at the incidence of HCC. *Conclusion:* HBcrAg is a predictor of the post-treatment recurrence of HCC during antiviral therapy. Serum HBcrAg and intrahepatic cccDNA suppression by NAs may be important to prevent HCC recurrence.

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently, and one million people die of decompensated cirrhosis and/or hepatocellular carcinoma (HCC) annually (1, 2). Recently, oral nucleot(s)ide analogues (NAs) have been used as the mainstay therapeutic strategy against chronic hepatitis B. Five such antiviral agents have been approved, and range in the profundity and rapidity of HBV DNA suppression, barrier to resistance and side-effect profile (3–10). Lamivudine (LAM) was the first NA to be approved for treating chronic hepatitis B, followed by adefovir dipivoxil (ADV) and entecavir (ETV), in Japan. However, a major problem with long-term LAM treatment is the potential development of drug resistance, mainly caused by mutation of the thyrosine–methionine–aspartic acid–aspartic acid (YMDD) motif of reverse transcriptase (11, 12). For preventing breakthrough hepatitis induced by LAM-resistant mutants, additional ADV administration has been recommended (13, 14).

The methods for monitoring the treatment response include measurements of the serum alanine transaminase (ALT) levels, HBV DNA levels, HBeAg and antibody levels, HBsAg and antibody levels and liver histology. Other serum markers have been reported to be useful for monitoring the effect of antiviral therapy (15, 16). Recently, a new assay was developed for detecting the HBcrAg, consisting of HBcAg, HBeAg and a 22 kDa precore protein coded with the precore/core gene (17, 18). Because NAs have no inhibiting action on the transcription and translation activities of viral mRNA, HBcAg- and HBeAg-related proteins continue to be produced for a certain period of time in spite of the achievement of adequate suppression of the viral DNA synthesis. Therefore, HBcrAg is a viral marker independent of HBV DNA for monitoring the antiviral effect of NAs (19). In addition, recent reports have indicated another interesting aspect of serum HBcrAg levels: these levels were found to be correlated with intrahepatic covalently closed circular DNA (cccDNA) levels and could be a surrogate marker of the intrahepatic cccDNA pool (20, 21). This phenomenon may be explained by the fact that the production of HBcrAg depends on the

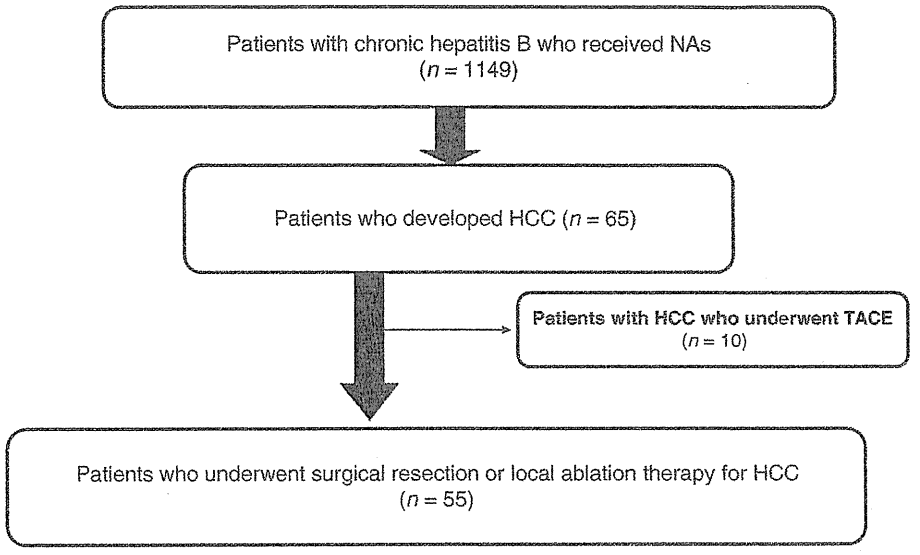

Fig. 1. The study protocol. HCC, hepatocellular carcinoma; NAs, nucleot(s)ide analogues; TACE, transcatheter arterial chemoembolization.

transcription of mRNA from cccDNA, and that cccDNA still remains in high levels during treatment with NAs.

Although patients with HBV-related cirrhosis have a significantly high risk of developing HCC, NA therapy can delay the progression of liver disease and reduce the risk of HCC in patients with cirrhosis by strong viral suppression (22, 23). Nevertheless, a few cases develop HCC during NA therapy at a constant rate (3–12%) (22, 24–26). The recurrence rate of HBV-related HCC after curative resection is estimated to be high, and is associated with viral factors, including HBeAg positivity and the viral load before surgery, besides host and tumour factors, but these findings were demonstrated in the absence of antiviral therapy (27–30). However, almost all patients, receiving NAs, showed negativity of serum HBV DNA. And so, we made the hypothesis that intrahepatic viral status, such as intrahepatic cccDNA and serum HBcrAg levels of its surrogate maker, might have an impact on tumour recurrence during NA therapy.

In this study, we examined the risk factors for tumour recurrence after curative resection and ablation for HBV-related HCC during NA therapy by measuring the serum HBcrAg and intrahepatic cccDNA levels with the aim to elucidate the viral status, persistent despite suppressive therapy, associated with HCC recurrence, in addition to the host and tumour factors reported in the past.

Patients and methods

Patients

Over a period of 13 years, from September 1995 to September 2008, 1149 patients with chronic hepatitis B received NA therapy, including LAM, ADV and ETV, at the Department of Hepatology, Toranomon Hospital, Metropolitan Tokyo. Of the 1149 patients, 65 developed HCC after the start of NA therapy from February 2001 to June 2009. Of the 65 consecutive patients, 55 underwent radical therapy, including either resection or percutaneous ablation as the initial therapy for HCC. These 55 patients were enrolled in this cohort study (Fig. 1). The median duration from the start of NA therapy to the development of HCC was 2.2 (range, 0.2–7.4) years. The exclusion criteria were (i) patients co-infected with hepatitis C, delta or human immunodeficiency virus and (ii) a history of other liver diseases such as autoimmune hepatitis, alcoholic liver disease or metabolic liver disease.

The diagnosis of HCC was predominantly based on imaging, including dynamic computed tomography, magnetic resonance imaging and/or digital subtraction angiography. When the hepatic nodule did not show the typical imaging features, fine needle aspiration biopsy was performed, followed by histological examination and diagnosis. The physicians and surgeons usually discussed the preferred choice of treatment for each patient. Hepatic resection was mainly performed for patients categorized as Child–Pugh grade A or B liver function, and had no serious complications. Percutaneous ablation was performed for patients with surgical contraindications or for those who did not prefer to undergo hepatic resection by using two different devices: the cool-tip system (Tyco Healthcare Group LP, Burlington, VT, USA) and the radiofrequency tumour coagulation system (RTC system; Boston-Scientific Japan Co., Tokyo, Japan). The term curative treatment was used to indicate that no tumours were left in the remnant liver, irrespective of the width of the margin around the tumour, confirmed using intra-operative ultrasonography, combined ultrasonography and dynamic computed tomography 1 month after the resection or ablation. Serum samples were collected from all patients before and after