Table 1 Pretreatment characteristics of chronic hepatitis C virus (CHCV) patients with HCV-1b RNA who received pegylated interferon α 2b + ribavirin standard therapy for 48 weeks

Characteristic	Value (n = 1480)
Sex (male/female)	898/582
Age (years)	57 (13–79)
History of HCC (yes/no/unknown)	8/1405/67
Previous IFN treatment (yes/no/unknown)	459/688/333
Diabetes (yes/no/unknown)	44/480/956
Hypertension (yes/no/unknown)	105/417/958
Ongoing alcohol use (yes/no/unknown)	157/456/867
Grade (A0/A1/A2/A3/unknown)	14/499/478/55/434
Stage (F0/F1/F2/F3/F4/unknown)	36/469/316/176/48/435
ALT (IU/L)	63 (8.4–910)
Platelets ($\times 10^4/\mu L$)	16.6 (4.3–47.7)
Viral load (KIU/mL)	1900 (100–5100)

Data expressed as median (range). HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; IFN, interferon.

received IFN. Whereas, multivariate logistic regression analysis revealed that older age (<55/≥55 years), degree of progression of hepatic fibrosis (F0–1/2–4), low platelet count (≥16/<16×10^4/μL), and high viral load (<1900/≥1900 KIU/mL) are resistance factors to SVR (Table 2). In multivariate logistic regression analysis, sex was not selected.

Study 2: usefulness of prolonged treatment in LVR patients

Of the patients who completed standard 48-week treatment, 223 patients (20.0%) showed LVR (Fig. 1), and median duration of treatment was 48 weeks. Compared with patients who exhibited early virologic response (EVR) defined as HCV-RNA negative within 12 weeks after the start of treatment, those with LVR were older (median age, 58 vs 55 years; $P = 0.0043$) and had higher viral load (median, 2700 vs 1620 KIU/mL; $P < 0.0001$) and lower platelet count (median, 16.5 vs 17.3×10^4/μL; $P = 0.0162$). SVR rate based on treatment analysis was 56.5 in all, 79.2% in EVR and 46.2% in LVR, respectively. In multivariate logistic regression analysis of SVR-related factors in LVR patients who completed standard 48-week treatment, age (10-year groups) was selected as a significant factor.

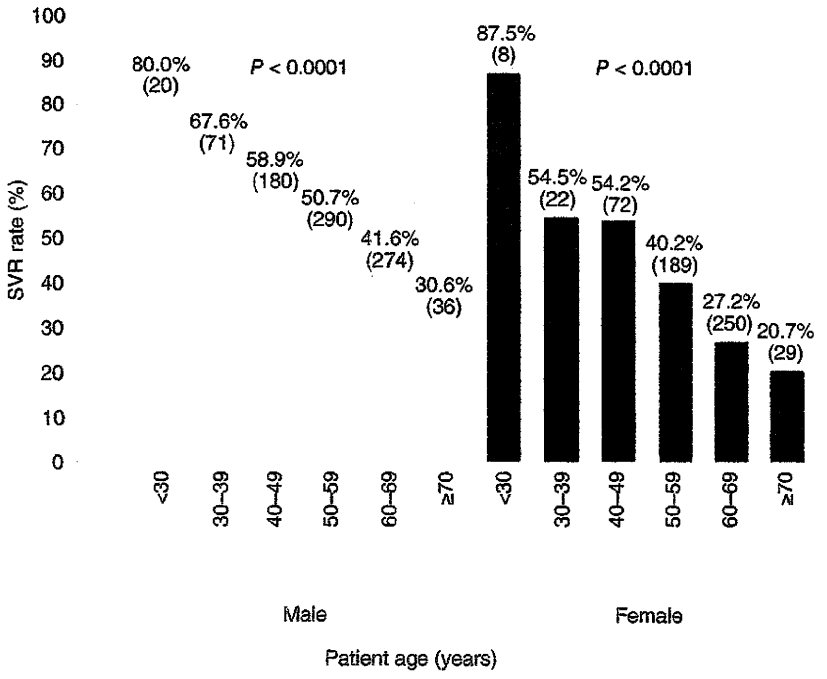

Figure 2 Sustained virological response (SVR) rate to 48 weeks' standard treatment with pegylated interferon α 2b (PEG-IFN α 2b) + ribavirin in male and female patients stratified by age. Cochran–Armitage test was used to study the underlying trend.

Table 2 Independent factors associated with sustained virological response in genotype 1 chronic hepatitis C virus patients who received pegylated interferon α 2b + ribavirin standard therapy for 48 weeks

	Odds ratio	95% confidence interval	*P*-value†
Age <55/≥55 years	0.414	0.293–0.585	<0.0001
Stage 0–1/2–4	0.633	0.442–0.906	0.0124
Platelets <16/≥16 × 10^4/μL	1.876	1.305–2.696	0.0007
Viral load </≥1900 KIU/mL	0.663	0.471–0.935	0.0192

†Multiple logistic regression analysis.

Prolonged treatment was conducted in 73 LVR patients (Fig. 1), with mean duration of 72 weeks. As shown in Table 3, whereas among LVR patients there were significantly ($P = 0.0061$) more female subjects in 72-week group than 48-week group, no intergroup difference of other factors was observed. Overall, SVR rate based on treatment analysis was significantly ($P = 0.0020$) higher in 72-week treatment group than in 48-week treatment group (67.1% [49/73] vs 46.2% [103/223]; Fig. 3A).

When stratified by sex, SVR rate with 48-week and 72-week treatment was 51.4% and 68.6% ($P = 0.0809$) in male subjects and 37.3% and 65.9% ($P = 0.0039$) in female subjects, with SVR in 72-week treatment being significantly higher in female subjects and indicating that, in LVR patients, efficacy comparable to male subjects is achieved in female subjects with 72-week treatment.

In patients aged <55 years SVR rate in the 48- and 72-week treatment groups was 57.6% and 78.9% ($P = 0.1100$) in male subjects and 40.0% and 76.9% ($P = 0.0724$) in female subjects, respectively, with higher SVR rates for the 72-week treatment group (Fig. 3B). In patients aged ≥55 years this parameter was 44.6% and 53.8% ($P = 0.5619$) in male subjects and 37.1% and 60.7% ($P = 0.0425$) in female subjects, respectively, with higher SVR rates for the 72-week treatment group than for the 48-week treatment group as in the case of the younger age group (Fig. 3C).

DISCUSSION

Study 1: SVR-related factors in patients receiving standard 48-week treatment

SVR RATE WITH standard 48-week treatment in this study was 44.9%, roughly equal to the 45% reported in previous clinical trials in Japan.[4,17–19] The present results are also similar to those of clinical trials conducted in patients aged in their mid-40s in western countries and in the general clinical setting.[1–4] Age was

Table 3 Comparison of clinical and virological characteristics between groups receiving pegylated interferon α 2b + ribavirin therapy for 48 and 72 weeks among patients showing late virological response

	48 weeks' group ($n = 223$)	72 weeks' group ($n = 73$)
Sex (male/female)	140/83*	32/41*
Age (years)	58 (21–75)	56 (22–71)
History of HCC (yes/no/unknown)	1/221/11	0/73/0
Previous IFN treatment (yes/no/unknown)	68/113/42	29/32/12
Diabetes (yes/no/unknown)	11/71/141	1/34/38
Hypertension (yes/no/unknown)	18/62/143	6/29/38
Ongoing alcohol use (yes/no/unknown)	17/75/131	6/27/40
Grade (A0/A1/A2/A3/unknown)	2/66/82/6/67	0/21/26/4/22
Stage (F0/F1/F2/F3/F4/unknown)	7/68/45/32/5/66	2/16/20/12/2/21
ALT (IU/L)	61.5 (14–550)	52 (17–254)
Platelets (×10^4/μL)	16.5 (8.5–43.2)	16.6 (4.3–40.2)
Viral load (KIU/mL)	2700 (160–5100)	2100 (130–5000)

Data expressed as median (range). *$P = 0.006$. ALT, alanine aminotransferase; HCC, hepatocellular carcinoma; IFN, interferon.

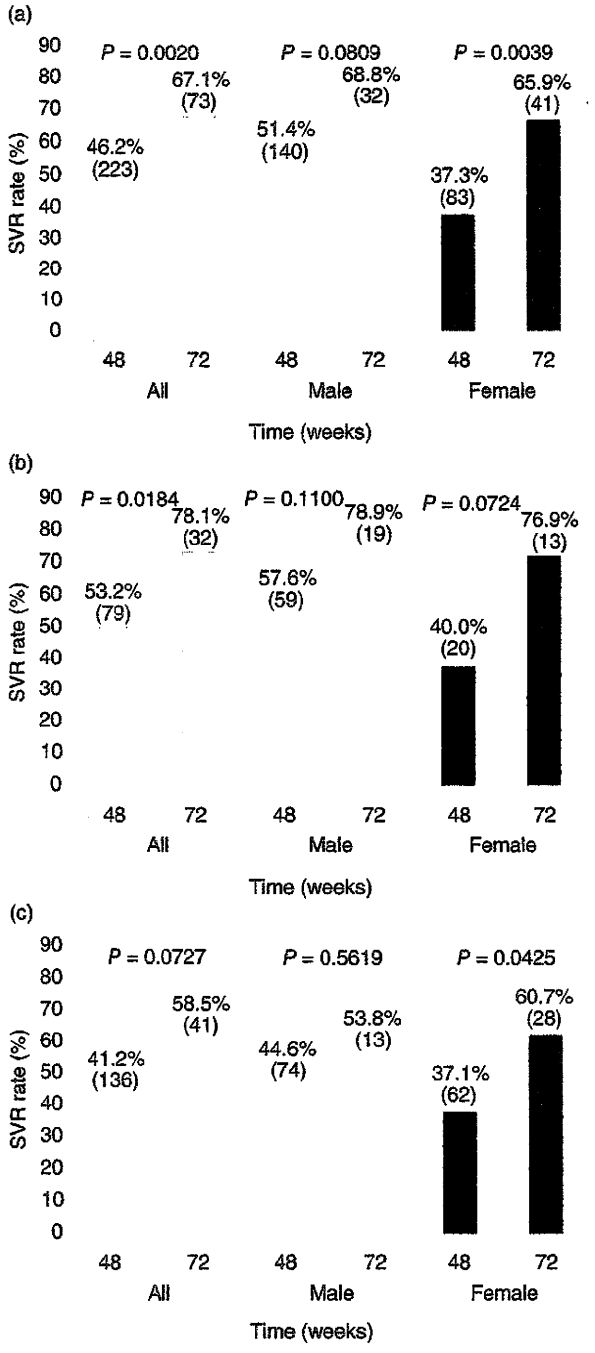

Figure 3 Sustained virological response (SVR) rate based on treatment analysis between groups receiving pegylated interferon α 2b (PEG-IFN α 2b) + ribavirin therapy for 48 and 72 weeks who exhibited late virological response (LVR). (A) Overall; (b) patients aged <55 years; (c) patients aged ≥55 years. Data on age not available for 7 male patients and 1 female patient.

selected among factors for SVR with PEG-IFN plus RBV combination therapy in an aging patient population, the examination of which was the objective of this study, and SVR rate decreased stepwise with 10-year age increase. Of particular note was the greater impact of aging observed in female than male subjects.

Lower efficacy in elderly female patients infected with HCV genotype 1 has already been reported in Japan.[20] A low SVR rate was also observed in elderly female subjects in this study. Although female sex was considered a favorable prognostic factor in some Western studies, there is no established opinion on sex difference. Change associated with aging of the patient population in Japan is considered to account for this phenomenon observed in the present study. This may be due to decrease in compliance among elderly women; on the other hand, however, there was no difference between male and female subjects aged ≥55 years in the rate of completion of treatment. Although the rate of dose reduction of RBV tended to be slightly higher in female subjects (data not shown), the difference was not significant. These findings suggest the influence of factors other than adherence to treatment for the low SVR rate among elderly women. One possible factor for reduced SVR rate among these individuals may be the effect of menopause. In women, insulin resistance begins to worsen after the age of 50 years,[21,22] and this is reported more closely associated with the effect of menopause than age itself.[23]

The presence of insulin resistance has been reported to lower efficacy of PEG-IFN and RBV combination therapy.[24–27] Insulin resistance is also a cause of advanced fibrosis and fatty change of the liver.[28–31] It is possible that such changes combined with other factors associated with metabolic syndrome interact in a complex way to reduce the efficacy of this therapy.[32–35] In fact, the incidence of non-alcoholic fatty liver disease (NAFLD) among elderly Asians was reported higher in women as compared with that in men.[36–38] However, while older age, advanced fibrosis, low platelet count and high HCV load were selected as factors for reduction of SVR rate in our multivariate logistic regression analysis, sex was not selected. It is therefore necessary to examine further the confounding of these selected factors with sex. It also should be taken into consideration that, due to limitations imposed by the retrospective nature of this study, data on factors affecting the efficacy of PEG-IFN plus RBV therapy such as insulin resistance, steatosis, and core mutation are lacking. A large-scale prospective study is

required to examine the lower efficacy observed in elderly women.

Study 2: usefulness of prolonged treatment in LVR patients

EVR (viral load reduced by 2 log or undetected in week 12) has been used for determining continuation or discontinuation of treatment in western countries. Recently, however, EVR was divided into complete EVR (HCV RNA <50 IU/mL at week 12) and partial EVR (>2 log drop in HCV RNA but still detectable [>50 IU/mL]). Fried *et al.*[15] and Berg *et al.*[16] reported that the SVR rate was a high 68–84% in patients showing complete EVR but only 17–29% in those with partial EVR with treatment for 48 weeks. They also reported that treatment for 72 weeks was effective in patients with partial EVR. In the clinical study for health registration in Japan, the SVR rate by timing of HCV-RNA negativity at 4, 12, and 24 weeks was 100%, 71.1%, and 36.4%, respectively, and no patient with HCV-RNA negativity after 25 weeks achieved SVR.[4] With these studies as reference, patients with LVR were defined as those who were positive (>50 IU/mL) at week 12 and became negative (<50 IU/mL) by week 24. To minimize the influence of treatment discontinuation, only patients who completed the standard duration of treatment were selected as subjects in this study. In the comparison of patient background, there was no significant intergroup difference except for a significantly greater number of female subjects in the 72-week treatment group. This finding might be related to the observation that it was already widely believed that efficacy in elderly women in Japan is low and that duration of treatment was at the discretion of individual physicians. Nevertheless, it is noteworthy that the SVR rate was significantly higher in the 72-week treatment group than in the 48-week treatment group and that a high 60% SVR rate was achieved with 72-week treatment in elderly female patients, a population in whom a relatively low SVR was observed with standard 48-week treatment.

This retrospective study had the limitation that duration of treatment was at the sole discretion of each participating physician. A prospective study is necessary to demonstrate whether 72-week treatment in elderly women with LVR is more efficaous than 48-week treatment in male patients. Although the number of younger subjects examined was rather low, it is noteworthy that an SVR rate of >75% was observed with 72-week treatment in both male and female patients. This also should be confirmed by prospective study.

CONCLUSIONS

PATIENTS WITH CHCV genotype 1 infection should be treated with PEG-IFN and ribavirin combination therapy as early as possible. Seventy-two weeks' treatment is recommended in patients with LVR, regardless of age.

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APPENDIX I

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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.

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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±[illegible]
FPG (mg/dL)	[illegible]
Triglyceride (mg/dL)	[illegible]
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	0.530
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.