

concentration decreased to less than 10 g/dL, and was discontinued when the Hb concentration decreased to less than 8.5 g/dL. Both Peg-IFN α -2b and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. No ferric medicine or haematopoietic growth factors, such as epoetin alpha, or granulocyte-macrophage colony stimulating factor, were administered.

Virologic assessment and definition of virologic response

Serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 kIU/mL; Roche Diagnostics, Branchburg, NJ, USA) and qualitatively analysed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/mL; Roche Diagnostics). Complete early virologic response (c-EVR) was defined as the absence of detectable serum HCV RNA at treatment week 12, the late virologic response (LVR) was defined as undetectable serum HCV RNA for the first time at 13–24 weeks of treatment, and the virologic response (VR) was defined as HCV RNA negativity at week 24 and week 48. SVR was defined as the absence of detectable serum HCV RNA at week 72. Patients with less than a 2-log decrease in HCV RNA level at treatment week 12 compared with the baseline had to stop treatment according to the protocol and were regarded as nonresponders. All patients with detectable serum HCV RNA at treatment week 24 were also considered to be nonresponders and were excluded from further treatment.

Assessment of drug exposure

The amounts of Peg-IFN α -2b and ribavirin actually taken by each patient during the full treatment period were evaluated by reviewing the medical records. The mean doses of Peg-IFN α -2b and ribavirin were calculated individually as averages on the basis of body weight at baseline: Peg-IFN α -2b expressed as μ g/kg/week, ribavirin expressed as mg/kg/day.

Evaluation of impact of drug exposure on virologic relapse

We evaluated the relationship between the drug exposure of both drugs and relapse by two different methods, univariate and multivariate analysis for relapse and independent evaluation of both drugs for relapse according to the degree of drug exposure. The former was performed with the factors of mean administration doses of both drugs, including the factors at baseline and the timing of HCV RNA negativation. The latter was examined by classifying Peg-IFN α -2b exposure into five categories (up to 0.6 μ g/kg; from 0.6 to less than 0.9 μ g/kg; from 0.9 to less than 1.2 μ g/kg; from 1.2 to less than 1.5 μ g/kg; from 1.5 μ g/kg) and ribavirin exposure into five categories (up to 6 mg/kg; from 6 to less than 8 mg/kg; from 8 to less than 10 mg/kg; from 10 to less than 12 mg/kg; from 12 mg/kg).

Statistical analysis

Baseline data are expressed as means \pm SD or median values. Virologic response was evaluated using per protocol (PP) analysis. To analyse the difference between baseline data including drug exposure and virologic response, univariate analysis using the Mann–Whitney *U*-test or chi-square test and multivariate analysis using logistic regression analysis were performed. The significance of trends in values was determined with the Mantel–Haenszel chi-square test. A two-tailed *P* value <0.05 was considered significant. The analysis was conducted with SPSS version 15.0J (SPSS Inc., Chicago, IL, USA).

RESULTS

Progress of patients and dose reduction of Peg-IFN α -2b and ribavirin

The progress of patients in this study is shown in Fig. 1. Of the 984 patients, 903 completed 12 weeks of treatment and the c-EVR rate was 49% (445/903), based on PP study. To analyse for relapse, 472 patients with VR were assessed, with 178 (38%) showing Peg-IFN dose reduction without discontinuation and 246 (52%) with ribavirin dose reduction without discontinuation during the full (48 weeks) treatment period. The relapse rate was 26% (125/472) in the patients with undetectable HCV RNA level at the end of treatment. No difference was found in relapse rates between the IFN naïve patients and IFN experienced patients (IFN naïve; 25%, 72/287 vs IFN experienced; 29%, 53/185, *P* = 0.40). The SVR rate was 43% (347/812) in the PP study.

Impact of drug exposure during 0–48 weeks on relapse among patients with VR

The mean dose of Peg-IFN α -2b actually taken during the full treatment period by each patient was 1.32 μ g/kg/week (range, 0.49–2.16 μ g/kg/week; median, 1.38 μ g/kg/week) and that of ribavirin was 9.8 mg/kg/day (range, 3.3–16.2 mg/kg/day; median, 10.1 mg/kg/day) in patients with VR.

The result of univariate analysis for relapse among the patients with VR is shown in Table 2a. The degree of fibrosis, the timing of HCV RNA negativation, Plt value and the mean doses of ribavirin were factors significantly associated with relapse, but those of Peg-IFN α -2b were not. The mean dose of ribavirin as well as the degree of fibrosis and the timing of HCV RNA negativation was selected as a significant independent factor by multivariate logistic regression analysis (Table 2b).

Next, we analysed the relationship of the relapse rate and the mean ribavirin dose. The overall relapse rate among patients with VR was 26% (125/472). The

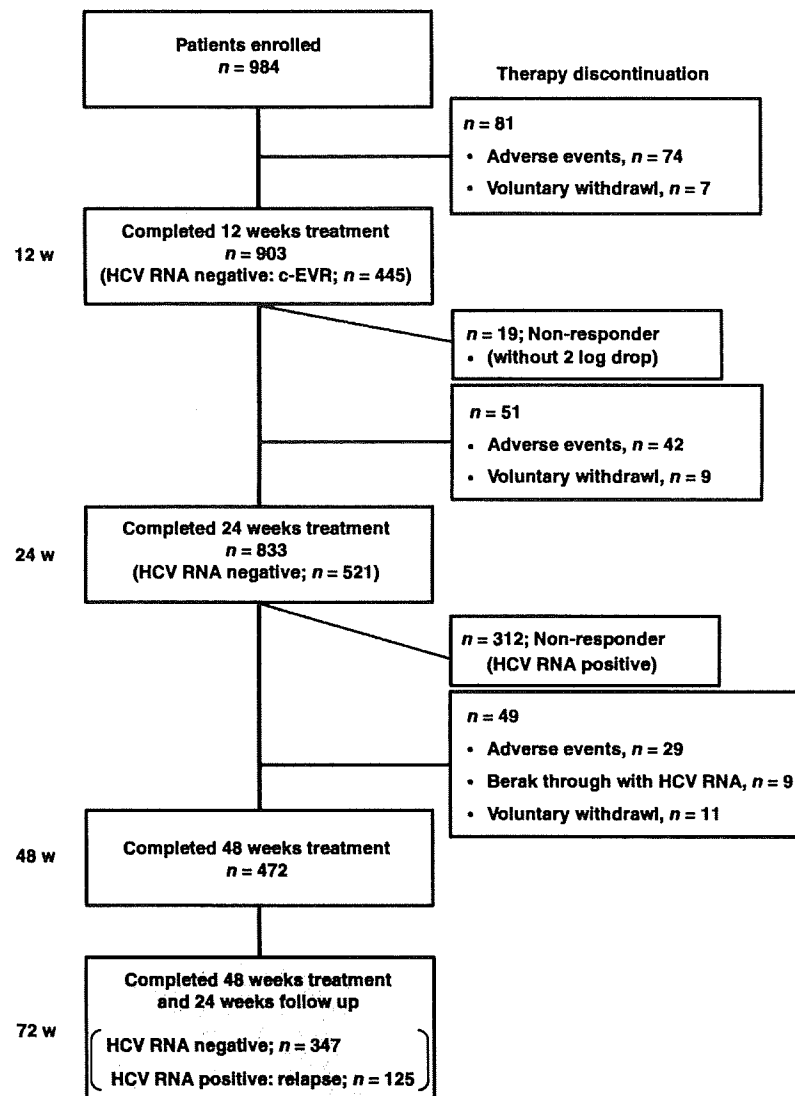


Fig. 1 Flow of patients throughout the study.

relapse rate was 60% (9/15) in patients receiving less than 6 mg/kg/day of ribavirin, and declined to 41% (32/79) at 6–8 mg/kg/day, 27% (34/124) at 8–10 mg/kg/day, 22% (43/193) at 10–12 mg/kg/day and 11% (7/61) in patients given ≥ 12 mg/kg/day ($P < 0.0001$). Figure 2 shows the relationship of the relapse rate and the mean ribavirin dose for two dosage groups of Peg-IFN α -2b: the group given ≥ 1.4 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN and that given < 1.4 $\mu\text{g}/\text{kg}/\text{week}$ (1.4 $\mu\text{g}/\text{kg}/\text{week}$ was the median value). In both groups, ribavirin was dose-dependently correlated with relapse. More than 12 mg/kg/day of the mean ribavirin exposure could suppress the relapse rate to 20% (4/20) in the group given < 1.4 $\mu\text{g}/\text{kg}/\text{week}$ and strongly suppress it to 7% (3/41) in the group given ≥ 1.4 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN.

Impact of drug exposure during 0–48 weeks on relapse according to the timing of HCV RNA negativation

Relapse rates among patients with c-EVR

The overall relapse rate among patients with c-EVR was 19% (75/391). We separately analysed the relapse rate among the patients with c-EVR according to the degree of exposure to both drugs. Table 3a shows the relapse rates among the patients with c-EVR according to the categories of Peg-IFN α -2b and ribavirin doses during the full treatment period. The relapse rate showed a decline according to the increase in the dose of ribavirin ($P = 0.0002$). The relapse rate was suppressed at an average of 15% (13–16%) in the patients who received 10–12 mg/kg/day of ribavirin, and the average was only 4% for those who received more than 12 mg/kg/day

Table 2 Factors associated with relapse among the patients with virologic response

(a) Univariate analysis				
Factor	Nonrelapser	Relapser	P value	
<i>n</i>	347	125		
Age (years)	53.9 ± 10.7	56.2 ± 9.2	0.07	
Sex (male/female)	213/134	66/59	0.09	
Serum HCV RNA (kIU/mL)*	1600	1800	0.34	
White blood cells (/mm ³)	5335 ± 1517	5075 ± 1428	0.08	
Neutrophils (/mm ³)	2797 ± 1143	2625 ± 1021	0.17	
Red blood cells (×10 ⁴ /mm ³)	450 ± 45	446 ± 50	0.25	
Haemoglobin (g/dL)	14.3 ± 1.4	14.2 ± 1.5	0.45	
Platelets (×10 ⁴ /mm ³)	17.6 ± 5.3	16.4 ± 5.1	0.03	
AST (IU/L)	60 ± 42	58 ± 33	0.75	
ALT (IU/L)	75 ± 60	71 ± 50	0.98	
Histology (METAVIR) [†]				
Fibrosis: 0–2/3–4	222/20	74/19	0.002	
Activity: 0–1/2–3	140/102	52/41	0.75	
Peg-IFN dose (µg/kg/week) [‡]	1.33 ± 0.26	1.27 ± 0.29	0.07	
Ribavirin dose (mg/kg/day) [‡]	10.1 ± 1.9	9.1 ± 2.1	<0.001	
Virologic response [§] : c-EVR/LVR	316/31	75/50	<0.001	
(b) Multivariate analysis				
Factor	Category	Odds ratio	95% CI	P value
Platelets	By 1 × 10 ⁴ /mm ³	–	–	NS
Fibrosis [¶]	0–2/3–4	1/3.192	1.515–6.725	0.002
Ribavirin dose [‡]	By 1 mg/kg/day	0.790	0.696–0.896	<0.001
Virologic response [§]	c-EVR/LVR	1/6.290	3.385–11.690	<0.001

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus; c-EVR, complete early virologic response; LVR, late virologic response; NS, not significant difference Peg-IFN, pegylated interferon.

*Data shown are median values. †137 missing. ‡Mean doses during 0–48 weeks. §The timing of HCV RNA negativation. ¶METAVIR fibrosis score.

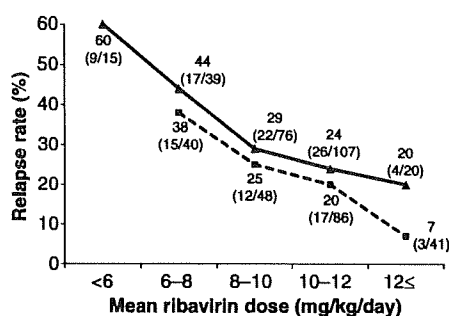


Fig. 2 Relapse rate according to Peg-IFN α -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses. (— \blacktriangle) Group with the mean Peg-IFN dose <1.4 $\mu\text{g}/\text{kg}/\text{week}$; (--- \blacksquare) Group with the mean Peg-IFN dose ≥ 1.4 $\mu\text{g}/\text{kg}/\text{week}$. The ribavirin dose was dose-dependently correlated with the virologic relapse in both groups ($P < 0.0001$). There was no significant difference between the two Peg-IFN α -2b-dose groups ($P = 0.17$).

of ribavirin. In contrast, the relapse rate was not affected by the dose of Peg-IFN α -2b when the patients were given more than 0.9 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN α -2b. On the other hand, with respect to patients with rapid virologic response (RVR) defined as the absence of detectable serum HCV RNA at treatment week 4 ($n = 41$), none showed relapse and all attained SVR irrespective of the dose of Peg-IFN α -2b or ribavirin (prevalence of patients: the mean dose of Peg-IFN α -2b; $<0.9 : 0.9\text{--}1.2 : 1.2\text{--}1.5 : 1.5$ $\mu\text{g}/\text{kg}/\text{week} \leq 7 : 17 : 34 : 42\%$, the mean dose of ribavirin; $<8 : 8\text{--}10 : 10\text{--}12 : 12$ $\text{mg}/\text{kg}/\text{day} \leq 15 : 24 : 41 : 20\%$).

Relapse rates among patients with LVR

Among the patients with LVR, the ribavirin exposure during treatment was also the factor correlated adversely with the relapse rate ($P = 0.03$). However, the overall relapse rate was 62% ($50/81$), which was much higher than that of the c-EVR patients ($P < 0.0001$) and 45% ($5/11$) of patients with LVR relapsed even in the group given more than 12 $\text{mg}/\text{kg}/\text{day}$ of the average ribavirin dose (Table 3b).

Table 3 Relapse rate according to Peg-IFN and ribavirin doses during week 0–48 for patients with c-EVR and LVR who completed 48 weeks of treatment

(a) C-EVR										
Peg-IFN dose ($\mu\text{g}/\text{kg}/\text{week}$) [†]	Ribavirin dose (mg/kg/day)*								Total	
	12 \leq	10–12		8–10		<8				
≥ 1.5	0%	(0/28)	13%	(4/31)	14%	(3/21)	29%	(5/17)	12%	(12/97)
1.2–1.5	20%	(2/10)	16%	(16/100)	25%	(16/65)	23%	(7/30)	20%	(41/205)
0.9–1.2	0%	(0/7)	13%	(2/15)	15%	(2/13)	38%	(6/16)	20%	(10/51)
<0.9	0%	(0/5)	15%	(2/13)	55%	(6/11)	44%	(4/9)	32%	(12/38)
Total	4%	(2/50)	15%	(24/159)	25%	(27/110)	31%	(22/72)	19%	(75/391)

(b) LVR										
Peg-IFN dose ($\mu\text{g}/\text{kg}/\text{week}$) [§]	Ribavirin dose (mg/kg/day) [†]								Total	
	12 \leq	10–12		8–10		<8				
≥ 1.5	43%	(3/7)	50%	(1/2)	100%	(2/2)	100%	(4/4)	67%	(10/15)
1.2–1.5		(1/1)	60%	(12/20)	29%	(2/7)	82%	(9/11)	62%	(24/39)
<1.2	33%	(1/3)	50%	(6/12)	60%	(3/5)	86%	(6/7)	59%	(16/27)
Total	45%	(5/11)	56%	(19/34)	50%	(7/14)	86%	(19/22)	62%	(50/81)

Peg-IFN, pegylated interferon; c-EVR, complete early virologic response; LVR, late virologic response.

* $P = 0.0002$ for comparison of the four ribavirin groups. [†] $P = 0.08$ for comparison of the four Peg-IFN groups. [‡] $P = 0.03$ for comparison of the four ribavirin groups. [§] $P = 0.57$ for comparison of the three Peg-IFN groups.

Impact of dose reduction after week 12 on relapse among patients with c-EVR

Among c-EVR patients with no or little reduction of Peg-IFN α -2b (the average dose $\geq 1.2 \mu\text{g}/\text{kg}/\text{week}$) during the first 12 weeks, no significant difference was found in the relapse rate between those whose average dose of Peg-IFN α -2b was reduced to 0.6–1.2 $\mu\text{g}/\text{kg}/\text{week}$ during 12–48 weeks (17%, 7/41) and those without reduction of Peg-IFN α -2b (average dose $\geq 1.2 \mu\text{g}/\text{kg}/\text{week}$) (18%, 53/295) ($P = 0.86$) (Table 4a). Reducing the dose of Peg-IFN α -2b after week 12 in patients in whom HCV RNA had already become undetectable before week 12 did not appear to adversely influence virologic relapse when the average dose of Peg-IFN α -2b was more than 0.6 $\mu\text{g}/\text{kg}/\text{week}$ during 12–48 weeks, irrespective of the mean dose of Peg-IFN α -2b during the first 12 weeks. On the other hand, the ribavirin dose reduction after week 12 tended to affect the relapse rate in patients given $\geq 10 \text{ mg}/\text{kg}/\text{day}$ of the ribavirin dose during the first 12 weeks (Table 4b).

Impact of drug exposure during 0–48 weeks on relapse among VR patients with advanced fibrosis

In the evaluation of the 39 patients with VR with progression of fibrosis or cirrhosis (METAVIR fibrosis score 3 or 4) enrolled in this study, ribavirin exposure during treatment significantly correlated with relapse (nonrelapser, $10.5 \pm 2.1 \text{ mg}/\text{kg}/\text{day}$ vs relapser, $8.8 \pm 2.3 \text{ mg}/\text{kg}/\text{day}$; $P = 0.007$). Among patients with advanced fibrosis (score 3–4),

the relapse rate in patients given $\geq 10 \text{ mg}/\text{kg}/\text{day}$ of the average ribavirin dose was significantly low (36%, 9/25) in comparison with that in patients given $< 10 \text{ mg}/\text{kg}/\text{day}$ of ribavirin (71%, 10/14) ($P = 0.048$).

DISCUSSION

Previous studies have suggested that reducing the ribavirin dose within the first 12–20 weeks of treatment in patients with HCV genotype 1 was associated with a decline of SVR [7,13,14]. However, Shiffman *et al.* [8] recently reported that reducing the mean dose of ribavirin during the first 20 weeks of treatment had little impact on relapse for patients with CH-C genotype 1 and that SVR may not be adversely affected as long as the total cumulative ribavirin dose remains above 60%. As the reason for the inconsistency in the impact of reducing ribavirin on the antiviral effect, it was suggested that sample sizes of the previous studies were insufficient to assess the impact of reducing the dose of ribavirin independent of Peg-IFN. However, in Shiffman's study, while the impact of reducing the dose of Peg-IFN or ribavirin on SVR was indeed closely examined independently of each other with a large sample size, the subjects were limited to patients with advanced fibrosis or cirrhosis and prior nonresponse to Peg-IFN \pm ribavirin who were enrolled in the Hepatitis Antiviral Long-term Treatment Against Cirrhosis (HALT-C) trial. Reddy *et al.* [15] analysed the drug exposure retrospectively for 569 CH-C patients with genotype 1 enrolled in clinical trials of Peg-IFN α -2a plus

Table 4 Relapse rate according to drug doses during week 0–12 and 12–48 for patients with c-EVR who completed 48 weeks of treatment

(a) Peg-IFN		12–48 weeks			
Peg-IFN dose (mean, µg/kg/week)		≥1.2	0.9–1.2	0.6–0.9	<0.6
0–12 weeks	≥1.2	18% (53/295)	17% (5/30)	18% (2/11)	(1/1)
	0.9–1.2	–	22% (4/18)	33% (4/12)	60% (3/5)
	<0.9	(0/1)	(0/1)	17% (2/12)	20% (1/5)
Total*		18% (53/296)	18% (9/49)	23% (8/35)	45% (5/11)

(b) Ribavirin		12–48 weeks			
Ribavirin dose (mean, mg/kg/day)		≥12	10–12	8–10	<8
0–12 weeks	≥12	4% (2/47)	13% (3/23)	13% (1/8)	33% (1/3)
	10–12	–	15% (18/123)	22% (12/54)	20% (5/25)
	8–10	–	(1/1)	26% (10/38)	26% (10/39)
	<8	–	–	–	40% (12/30)
Total†		4% (2/47)	15% (22/147)	23% (23/100)	29% (28/97)

c-EVR, complete early virologic response; Peg-IFN, pegylated interferon.

* $P = 0.18$ for comparison of the four Peg-IFN groups. † $P < 0.0001$ for comparison of the four ribavirin groups.

ribavirin, and concluded that SVR was not affected adversely by ribavirin reduction unless the cumulative ribavirin exposure was less than 60%. This supported Shiffman's data, but in Reddy's study, the stepwise reduction in ribavirin dose was shown to be associated with a stepwise increase in relapse rate from 19% to 54%. Thus, the impact of ribavirin drug exposure on the antiviral effect (relapse) in patients with CH-C genotype 1 remains unclear. Further examination is needed to determine whether or not ribavirin can be reduced to a certain degree without adversely affecting virologic relapse or SVR in Peg-IFN and ribavirin combination therapy for CH-C genotype 1.

In order to raise the SVR rate in patients with genotype 1, two strategies are possible: one is enhancing the virologic response of HCV RNA negativity and another is reducing relapse. In Peg-IFN plus ribavirin treatment, raising the doses of either or both drugs (dose-up strategy) is the only way to enhance the virologic response of HCV RNA negativity, but this is always accompanied by a high risk and the discontinuation rate can increase with the dose-up of drug, although the virologic response among patients completing the therapy can be improved [16,17]. Therefore, in this study, we tried to manage the drug dose to reduce relapse in virologic responders with HCV RNA negativity. Large-scale clinical trials [1,2,9–12] have revealed that adding ribavirin to IFN or Peg-IFN monotherapy for patients with CH-C reduced the relapse rate from approximately 50% to under 20%. Bronowicki *et al.* [18] examined the effect of ribavirin on CH-C genotype 1 in Peg-IFN α -2a plus ribavirin treatment

by randomizing patients with HCV RNA negativity by week 24 into two groups, one continuing with ribavirin and the other receiving Peg-IFN α -2a alone after week 24. As a result, the virologic responders who stopped ribavirin treatment at week 24 were found to have a significantly higher rate of breakthroughs during therapy and higher relapse rates after therapy in comparison with those who received Peg-IFN plus ribavirin for the full treatment period (relapse rate; 42% vs. 29%, $P = 0.02$). These findings indicate that ribavirin plays a very important role in reducing relapse. However, the relationship between ribavirin dose and relapse rate has not been examined in detail. Considering that ribavirin has little influence on HCV RNA negativation [1,2,9–12], its dose impact on the antiviral effect should be carefully examined, not for the SVR rate of all patients, but for the relapse rate of patients responding to Peg-IFN plus ribavirin, as evaluating of ribavirin by SVR including HCV RNA negativation cannot differentiate it from the strong influence of the Peg-IFN effect, which affects HCV RNA negativation dose-dependently [19]. Here, we examined the correlation between the average dose of drugs and the virologic relapse for patients responding to the treatment.

We performed univariate and multivariate analysis for relapse among the factors of mean administration doses of both drugs, including baseline factors and the timing of HCV RNA negativation. We found exposure to ribavirin dose, timing of HCV RNA negativation and the degree of liver fibrosis to be the independent factors affecting the virologic relapse in patients with VR. This indicates that management

of the ribavirin dose, which is the variable factor, unlike baseline factors, plays an important role in suppressing the virologic relapse in patients with CH-C genotype 1 treated by Peg-IFN plus ribavirin treatment. This suggests that maintaining the ribavirin dose should lower the relapse rate even in patients with advanced fibrosis who are liable to relapse. In fact, among patients with advanced fibrosis (METAVIR score 3–4), the relapse rate in those given ≥ 10 mg/kg/day of the average ribavirin dose was significantly lower than that in patients given < 10 mg/kg/day of ribavirin (36% vs. 71%). However, the sample size was too small for subsequent analysis with stratification. Further study is needed to clarify the impact of ribavirin dose on viral relapse in patients with progression of fibrosis.

The relapse rate among patients with c-EVR showed a decline according to the increase in ribavirin dose during treatment week 0–48 and was not affected by the Peg-IFN α -2b dose when the patients were given more than 0.9 μ g/kg/week of Peg-IFN α -2b. Among the patients with c-EVR, none with RVR had a relapse and all attained SVR irrespective of the dose of Peg-IFN α -2b or ribavirin. Examination of the impact of dose reduction after week 12 on relapse among patients with c-EVR showed that the ribavirin dose reduction after week 12 tended to affect the relapse rate in patients given ≥ 10 mg/kg/day of the ribavirin dose during the first 12 weeks, while the Peg-IFN α -2b dose after week 12 could be reduced without any increase in relapse rate in patients given more than 0.6 μ g/kg/week of the average dose of Peg-IFN α -2b. On the other hand, maintaining the ribavirin did not lead to reduce the relapse rate in patients with LVR. About half relapsed even when given ≥ 12 mg/kg/day of the average ribavirin dose. This suggested that the relapse rate could not be reduced by management of the ribavirin dose in patients with LVR. Extended therapy should be chosen in LVR patients as shown in the previous studies [20–23].

Shiffman *et al.* [24] recently reported that maintaining the Hb level with epoetin alpha did not enhance SVR if ribavirin was started at the standard dose (800–1400 mg/day, mean dose 13.3 mg/kg/day), although discontinuance and the reduction rates of ribavirin were decreased and a higher mean dose of ribavirin was administered in comparison with those treated with Peg-IFN plus ribavirin without epoetin. If these findings apply to patients with CH-C genotype 1, this would suggest that the ribavirin dose does not need to be maintained during treatment with Peg-IFN plus ribavirin, which would not agree with our findings. However, closer examination of the Shiffman *et al.* study shows that Peg-IFN plus a higher dose of ribavirin (1000–1600 mg/day, mean dose 15.2 mg/kg/day) with epoetin was found to suppress the relapse rate and enhance SVR. These data agree with ours with respect to the point that higher doses of ribavirin are associated with a lower relapse rate. What differs is the ribavirin dose needed to suppress the relapse. This is likely to be due to ethnic differences between the subjects. In Shiffman's study, approximately 40% were African-Ameri-

cans in whom the virologic response is well established as being significantly lower than those of other ethnic groups [25,26], while in our study, all subjects were Japanese. In the African-Americans treated with Peg-IFN plus standard-dose ribavirin, the relapse rate (calculated from 48% of ETR and 19% of SVR) was 60%, while 18% relapse (from 38% of ETR and 31% of SVR) occurred in those given Peg-IFN plus high-dose ribavirin. The relapse rate of patients with c-EVR in our study was 19%, which was very close to that for those with Peg-IFN plus high-dose ribavirin in Shiffman's study. Ribavirin does not have a direct antiviral action against HCV [27,28], and is considered to play an important role in accelerating HCV-infected cell clearance [29] and eradicating them completely when an immune response against infected cells is induced by IFN or Peg-IFN [30,31]. Therefore, the difference between patients who are easy or difficult to treat due to ethnic differences or differences in response to Peg-IFN can result in the need for different doses of ribavirin to suppress the relapse rate in patients with CH-C genotype 1.

In conclusion, our results have demonstrated that ribavirin is dose-dependently correlated with a relapse in patients with CH-C genotype 1 responding to Peg-IFN plus ribavirin. Maintaining a high dose (≥ 12 mg/kg/day) of ribavirin during the full treatment period could strongly suppress the relapse in such patients, while Peg-IFN α -2b could be reduced without affecting relapse in patients with c-EVR. This possibility should be explored in a prospective study.

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Pegylated interferon alpha-2b (Peg-IFN α -2b) affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with Peg-IFN α -2b plus ribavirin

T. Oze,^{1,*} N. Hiramatsu,^{1,*} T. Yakushijin,¹ M. Kurokawa,¹ T. Igura,¹ K. Mochizuki,¹ K. Imanaka,² A. Yamada,³ M. Oshita,⁴ H. Hagiwara,⁵ E. Mita,⁶ T. Ito,⁷ Y. Inui,⁸ T. Hijioka,⁹ S. Tamura,¹⁰ H. Yoshihara,¹¹ E. Hayashi,¹² A. Inoue,¹³ Y. Imai,¹⁴ M. Kato,¹⁵ Y. Yoshida,¹ T. Tatsumi,¹ K. Ohkawa,¹ S. Kiso,¹ T. Kanto,¹ A. Kasahara,¹ T. Takehara¹ and N. Hayashi¹

¹Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Yamadaoka, Suita, Osaka, Japan; ²Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Osaka, Japan; ³Sumitomo Hospital, Osaka, Osaka, Japan; ⁴Osaka Police Hospital, Osaka, Osaka, Japan; ⁵Higashiosaka City Central Hospital, Higashiosaka, Osaka, Japan; ⁶National Hospital Organization Osaka National Hospital, Osaka, Osaka, Japan; ⁷Kansai Rousai Hospital, Amagasaki, Hyogo, Japan; ⁸Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Hyogo, Japan; ⁹National Hospital Organization Osaka Minami Medical Center, Kawachinagano, Osaka, Japan; ¹⁰Minoh City Hospital, Minoh, Osaka, Japan; ¹¹Osaka Rousai Hospital, Sakai, Osaka, Japan; ¹²Kinki Central Hospital of Mutual Aid Association of Public School Teachers, Itami, Hyogo, Japan; ¹³Osaka General Medical Center, Osaka, Osaka, Japan; ¹⁴Ikeda Municipal Hospital, Ikeda, Osaka, Japan; and ¹⁵National Hospital Organization Minami Wakayama Medical Center, Tanabe, Wakayama, Japan

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SUMMARY. Chronic hepatitis C (CH-C) genotype 1 patients who achieved early virologic response have a high probability of sustained virologic response (SVR) following pegylated interferon (Peg-IFN) plus ribavirin therapy. This study was conducted to evaluate how reducing drug doses affects complete early virologic response (c-EVR) defined as hepatitis C virus (HCV) RNA negativity at week 12. Nine hundred eighty-four patients with CH-C genotype 1 were enrolled. Drug doses were evaluated independently on a body weight base from doses actually taken. From multivariate analysis, the mean dose of Peg-IFN α -2b during the first 12 weeks was the independent factor for c-EVR ($P = 0.02$), not ribavirin. The c-EVR rate was 55% in patients receiving ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN, and declined to 38% at 0.9 – 1.2 $\mu\text{g}/\text{kg}/\text{week}$, and 22% in patients given < 0.9 $\mu\text{g}/\text{kg}/\text{week}$ ($P < 0.0001$). Even with stratified analysis according to

ribavirin dose, the dose-dependent effect of Peg-IFN on c-EVR was observed, and similar c-EVR rates were obtained if the dose categories of Peg-IFN were the same. Furthermore, the mean dose of Peg-IFN during the first 12 weeks affected HCV RNA negativity at week 24 ($P < 0.0001$) and SVR ($P < 0.0001$) in a dose-dependent manner. Our results suggest that Peg-IFN was dose-dependently correlated with c-EVR, independently of ribavirin dose. Thus, maintaining the Peg-IFN dose as high as possible during the first 12 weeks can yield HCV RNA negativity and higher c-EVR rates, leading to better SVR rates in patients with CH-C genotype 1.

Keywords: chronic hepatitis C, drug dose, early virologic response, HCV RNA negativity, pegylated interferon plus ribavirin, sustained virologic response.

Abbreviations: c-EVR, complete EVR; CH-C, chronic hepatitis C; EVR, early virologic response; G-CSF, granulocyte-macrophage colony stimulating factor; Hb, haemoglobin; HCV, hepatitis C virus; Peg-IFN, pegylated interferon; Plt, platelet; SVR, sustained virologic response; WBC, white blood cell.

Correspondence: Naoki Hiramatsu, MD, PhD, Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita City, Osaka 565-0871, Japan. E-mail hiramatsu@gh.med.osaka-u.ac.jp

*These authors contributed equally to this work.

INTRODUCTION

Pegylated interferon (Peg-IFN) plus ribavirin therapy can improve anti-viral efficacy for patients with chronic hepatitis C [1–5], and the prognosis of patients in whom hepatitis C virus (HCV) is successfully eradicated improves markedly [6–10]. However, HCV still persists in approximately half of genotype 1 patients treated with Peg-IFN plus ribavirin [2–4]. Therefore, the treatment method needs to be well managed in order to maximize the virologic response in these patients with HCV genotype 1.

In order to achieve sustained virologic response (SVR), earlier virologic response is very important for patients with chronic hepatitis C (CH-C) genotype 1. A high SVR rate (65–72%) was found in patients who achieved early virologic response (EVR) defined as a 2-log decrease in HCV RNA level at week 12, but only 0–3% SVR was seen in patients without EVR [3,11]. Additionally, complete EVR (c-EVR), which means HCV RNA negativity at week 12, is more strongly related to SVR [3].

The relationship between drug exposure and anti-viral effect has been reported in several papers [2,11–15]. McHutchison *et al.* [12] demonstrated that the SVR rate in patients who received $\geq 80\%$ of their total planned doses of Peg-IFN and ribavirin for $\geq 80\%$ of the scheduled duration of therapy was significantly higher than that of patients who received $< 80\%$ of one or both drugs (51% vs 34%) and also suggested that the impact of dose reduction was greatest in patients for whom the dose had to be decreased within the first 12 weeks of treatment. In a subsequent analysis, reducing the dose of Peg-IFN and ribavirin to $< 80\%$ of the full planned dose within the first 12 weeks was reported to reduce EVR rate from 80 to 33% [11]. Thus, drug adherence during the first 12 weeks has been shown to be very important for attaining EVR and SVR, but it remains obscure whether either drug can be reduced to a certain degree without adversely affecting the treatment efficacy.

In the present study, we examined the correlation between c-EVR and drug doses which are evaluated on a body weight basis from drug doses actually taken, in order to clarify the necessary drug exposure of Peg-IFN and ribavirin for achieving a higher c-EVR rate in patients with CH-C genotype 1.

PATIENTS AND METHODS

Patients

The current study was a retrospective, multicenter trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 984 patients with CH-C treated with a combination of Peg-IFN α -2b plus ribavirin were enrolled in this study between December 2004 and September 2006. The baseline characteristics of the patients are summarized in Table 1. All patients were Japanese, their mean age was 56.3 ± 10.1 years, and 56% were males. The mean serum alanine aminotransferase level was 79 ± 61 IU/L.

Patients eligible for this study were those who were infected with HCV genotype 1 and had a viral load of more than 10^5 IU/mL, but were negative for hepatitis B surface antigen or anti-human immunodeficiency virus. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis). Informed consent was obtained from each patient included in this study. This study was conducted according to the ethical guidelines of the 1975 Dec-

Table 1 Baseline characteristics of patients

Factor	Mean \pm SD or number
<i>n</i>	984
Age (year)	56.3 ± 10.1
Sex: male/female	555/429
Body weight (kg)	61.8 ± 11.5
History of interferon treatment	
Naïve/experienced	575/409(160/182)
(relapser/nonresponder)*	
White blood cells (per mm ³)	5052 ± 1550
Neutrophils (per mm ³)	2577 ± 1092
Red blood cells ($\times 10^4$ /mm ³)	442 ± 47
Haemoglobin (g/dL)	14.1 ± 1.4
Platelets ($\times 10^4$ /mm ³)	15.9 ± 5.5
AST (IU/L)	66 ± 45
ALT (IU/L)	79 ± 61
Serum HCV RNA (kIU/mL) [†]	1600
Histology (METAVIR) [‡]	
Fibrosis; 0/1/2/3/4	49/314/197/105/18
Activity; 0/1/2/3	23/329/304/27

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

*Viral response to previous treatment was unknown in 57 patients, and 10 patients had discontinued treatment. [†]Data shown are median values. [‡]301 missing.

laration of Helsinki and informed consent was obtained from each patient.

Treatment

All patients received Peg-IFN α -2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL; Schering-Plough) for the duration of the study of 48 weeks. Peg-IFN α -2b was given subcutaneously once weekly at a dosage of 60–150 μ g/kg based on body weight (body weight 35–45 kg, 60 μ g; 46–60 kg, 80 μ g; 61–75 kg, 100 μ g; 76–90 kg, 120 μ g; 91–120 kg, 150 μ g) and ribavirin was given orally twice a day at a total dose of 600–1000 mg/day based on body weight (body weight ≤ 60 kg, 600 mg; 60–80 kg, 800 mg; > 80 kg, 1000 mg), according to a standard treatment protocol for Japanese patients.

Dose reduction

Dose modification followed, as a rule, the manufacturer's drug information according to the intensity of the haematological adverse effects. The dose of Peg-IFN α -2b was reduced to 50% of the assigned dose if the white blood cell (WBC) count declined to < 1500 /mm³, the neutrophil count to < 750 /mm³ or the platelet (Plt) count to $< 8 \times 10^4$ /mm³, and was discontinued if the WBC count declined to < 1000 /

mm³, the neutrophil count to <500/mm³ or the Plt count to <5 × 10⁴/mm³. Ribavirin was also reduced from 1000 to 600 mg, or 800 to 600 mg, or 600 to 400 mg if the haemoglobin (Hb) level decreased to <10 g/dL, and was discontinued if the Hb level decreased to <8.5 g/dL. Both Peg-IFN α -2b and ribavirin had to be discontinued if there was a need to discontinue one of the drugs. During this therapy, ferric medicine or haematopoietic growth factors, such as erythropoietin alpha, or granulocyte-macrophage colony stimulating factor (G-CSF), were not administered.

Virologic assessment and definition of virologic response

Serum HCV RNA level was quantified using the COBAS AMPLICOR HCV MONITOR test, version 2.0 (detection range 6–5000 kIU/mL; Roche Diagnostics, Branchburg, NJ, USA) and qualitatively analysed using the COBAS AMPLICOR HCV test, version 2.0 (lower limit of detection 50 IU/mL). The c-EVR was defined as the absence of detectable serum HCV RNA at treatment week 12, and SVR was defined as the absence of detectable serum HCV RNA at week 72. Patients with less than a 2-log decrease in HCV RNA level at treatment week 12 compared with the baseline had to stop treatment and were regarded as nonresponders. All patients with detectable serum HCV RNA at treatment week 24 were also considered nonresponders and excluded from further treatment.

Assessment of drug exposure

The amounts of Peg-IFN α -2b and ribavirin actually taken by each patient during the first 12 weeks of the treatment were evaluated by reviewing the medical records. The mean doses of both drugs were calculated individually as averages on the basis of body weight at baseline: Peg-IFN α -2b expressed as μ g/kg/week, and ribavirin expressed as mg/kg/day.

Evaluation of impact of drug exposure on c-EVR

We evaluated the relationship between the drug exposure of both drugs and c-EVR by univariate and multivariate analysis for c-EVR, using the factors of mean administration doses of both drugs during the first 12 weeks and the factors at baseline. Furthermore, Peg-IFN α -2b dose (average dose per body weight and per week) was classified into five categories (up to 0.6 μ g/kg; from 0.6 to <0.9 μ g/kg; from 0.9 to <1.2 μ g/kg; from 1.2 to <1.5 μ g/kg; from 1.5 μ g/kg and above). Ribavirin exposure was classified into four categories (up to 8 mg/kg; from 8 to <10 mg/kg; from 10 to <12 mg/kg; from 12 mg/kg and above), in order to examine the impact of Peg-IFN dose exposure on c-EVR. This impact was also evaluated based on the percentage of the total prescribed dose and compared with that based on the mean dose per body weight.

Statistical analysis

Baseline data for various demographic, biochemical and virologic characteristics of the patients are expressed as mean \pm SD or median values. To analyse the relationship between baseline data including drug exposure and c-EVR, univariate analysis using the Mann–Whitney *U*-test or chi-squared test and multivariate analysis using logistic regression analysis were performed. The significance of trends in values was determined with the Mantel–Haenszel chi-square test. A two-tailed *P*-value < 0.05 was considered significant. Statistical analysis was conducted with SPSS version 15.0J (SPSS Inc., Chicago, IL, USA).

RESULTS

Progress of patients treated with Peg-IFN α -2b and ribavirin

Of the 984 patients, 81 discontinued treatment because of adverse events ($n = 74$) or voluntary withdrawal ($n = 7$) by treatment week 12. The 903 patients who completed 12 weeks of treatment were assessed for c-EVR. During 12–48 weeks of treatment, 331 of the nonresponders and nine of breakthrough discontinued treatment, as did 91 patients (adverse events, $n = 71$; voluntary withdrawal, $n = 20$). A total of 472 patients completed 48 weeks of treatment.

Drug reduction and virologic response

Peg-IFN α -2b was reduced without discontinuation in 29% ($n = 266$) and ribavirin was reduced without discontinuation in 40% ($n = 359$) of the 903 patients who completed 12 weeks of treatment. The c-EVR rate was 49% (445/903) and HCV RNA was negative at week 24 in 60% (542/903) of patients who completed 12 weeks of treatment. Of the 445 patients with c-EVR, 327 patients achieved SVR (73%). Only 7% of the 458 patients without c-EVR did so.

Impact of dose exposure of Peg-IFN α -2b and ribavirin on c-EVR

The mean dose of Peg-IFN α -2b actually taken during the first 12 weeks by each patient was 1.33 μ g/kg/week (range 0.41–2.16 μ g/kg/week; median 1.40 μ g/kg/week) and that of ribavirin was 10.4 mg/kg/day (range 2.9–16.2 mg/kg/day; median 10.6 mg/kg/day).

The mean doses of both drugs and the factors at baseline correlated with the c-EVR were assessed by univariate and multivariate logistic regression analyses. Univariate analysis showed that factors significantly associated with c-EVR were age, sex, WBC, neutrophils, red blood cells, Hb, Plt, aspartate aminotransferase, the degree of liver fibrosis and the mean doses of Peg-IFN α -2b and ribavirin during the first 12 weeks (Table 2). The factors selected as significant by the univari-

Table 2 Univariate analysis for c-EVR among patients who completed 12 weeks treatment

Factor	c-EVR (+)	c-EVR (-)	P-value
<i>n</i>	445	458	
Age (year)	54.4 ± 10.4	57.5 ± 9.6	<0.001
Sex: male/female	267/178	237/221	0.01
Serum HCV RNA (kIU/mL)*	1500	1600	0.28
White blood cells (per mm ³)	5336 ± 1536	4818 ± 1547	<0.001
Neutrophils (per mm ³)	2789 ± 1133	2398 ± 1038	<0.001
Red blood cells (×10 ⁴ /mm ³)	450 ± 46	435 ± 49	<0.001
Haemoglobin (g/dL)	14.3 ± 1.4	13.9 ± 1.4	<0.001
Platelets (×10 ⁴ /mm ³)	17.3 ± 5.2	15.0 ± 5.6	<0.001
AST (IU/L)	62 ± 44	69 ± 44	<0.001
ALT (IU/L)	77 ± 64	80 ± 57	0.07
Histology (METAVIR) [†]			
Fibrosis: 0–2/3–4	273/37	247/74	<0.001
Activity: 0–1/2–3	171/139	159/162	0.16
Peg-IFN dose (µg/kg/week) [‡]	1.39 ± 0.22	1.28 ± 0.30	<0.001
Ribavirin dose (mg/kg/day) [‡]	10.6 ± 1.7	10.1 ± 2.1	0.002

c-EVR, complete early virologic response; HCV, hepatitis C virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Peg-IFN, pegylated interferon.

*Data shown are median values. [†]272 missing. [‡]Mean doses during 0–12 weeks.

Table 3 Multivariate analysis for c-EVR among patients who completed 12 weeks treatment

Factor	Category	Odds ratio	95% CI	P-value
Age	by 1 year	0.982	0.966–0.999	0.04
Sex	male/female	–	–	NS
Neutrophils	by 100/mm ³	1.017	1.002–1.033	0.03
Red blood cells	by 1 × 10 ⁴ /mm ³	–	–	NS
Haemoglobin	by 1 g/dL	–	–	NS
Platelets	by 1 × 10 ⁴ /mm ³	1.051	1.014–1.088	<0.01
AST	by 1 IU/L	–	–	NS
Fibrosis*	0–2/3–4	–	–	NS
Peg-IFN dose [†]	by 0.1 µg/kg/week	1.079	1.011–1.151	0.02
Ribavirin dose [†]	by 1 mg/kg/day	–	–	NS

95% CI, 95% confidence interval; Peg-IFN, c-EVR, complete early virologic response; pegylated interferon; N.S., No Significant difference; AST, aspartate aminotransferase.

*METAVIR fibrosis score. [†]Mean doses during 0–12 weeks.

ate analysis were evaluated by multivariate logistic regression analysis. The mean dose of Peg-IFN α -2b during the first 12 weeks was the independent factor for c-EVR ($P = 0.02$), apart from the neutrophils ($P = 0.03$) and Plt value at baseline ($P < 0.01$) and age ($P = 0.04$) (Table 3). In contrast, the mean dose of ribavirin during the first 12 weeks showed no correlation with c-EVR.

The c-EVR rates were 54% (137/253) and 56% (246/443) for patients who received ≥ 1.5 and 1.2–1.5 µg/kg/week of Peg-IFN α -2b on average during the first 12 weeks, and declined to an average rate of 38% (40/105) in patients given 0.9–1.2 µg/kg/week of Peg-IFN α -2b, and an average rate of 22% (22/102) in patients given < 0.9 µg/kg/week ($P < 0.0001$) (Table 4). The c-EVR rate among the patients

with ≥ 1.2 µg/kg/week of Peg-IFN α -2b was significantly higher than that of the patients with < 1.2 µg/kg/week [≥ 1.2 µg/kg/week, 55% (383/696) vs < 1.2 µg/kg/week, 30% (62/207), $P < 0.0001$].

Next, we analysed the impact of Peg-IFN α -2b on c-EVR in stratified analysis according to ribavirin dose. Figure 1 shows the relationship of c-EVR and the degree of Peg-IFN α -2b exposure for two groups of ribavirin doses: the group with ≥ 10.6 mg/kg/day of ribavirin and that with < 10.6 mg/kg/day (10.6 mg/kg/day was the median value). In either group, the mean dose of Peg-IFN α -2b was dose-dependently correlated with c-EVR ($P < 0.0001$), and c-EVR rates were very similar in both groups if the dose categories of Peg-IFN α -2b were the same.

Table 4 The c-EVR rate according to Peg-IFN and ribavirin doses during weeks 0–12 for patients who completed 12 weeks treatment

Ribavirin dose (mg/kg/day)**	Peg-IFN α -2b dose (μ g/kg/week),*				Total
	≥ 1.5	1.2–1.5	0.9–1.2	<0.9	
≥ 12	57% (60/105)	61% (22/36)	38% (6/16)	22% (2/9)	54% (90/166)
10–12	54% (46/85)	58% (154/267)	36% (14/39)	23% (11/47)	51% (225/438)
8–10	50% (25/50)	53% (52/99)	52% (15/29)	18% (4/22)	48% (96/200)
<8	46% (6/13)	44% (18/41)	24% (5/21)	21% (5/24)	34% (34/99)
Total	54% (137/253)	56% (246/443)	38% (40/105)	22% (22/102)	49% (445/903)

c-EVR, complete early virologic response; Peg-IFN, pegylated interferon.

* $P < 0.0001$ for comparison of the four Peg-IFN groups. ** $P = 0.05$ for comparison of the four ribavirin groups.

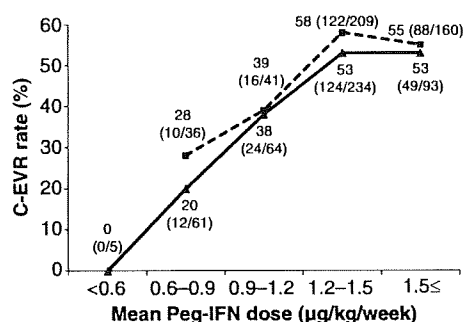


Fig. 1 Complete-EVR rate according to pegylated interferon alpha-2b (Peg-IFN α -2b) and ribavirin doses during weeks 0–12 for patients who completed 12 weeks of treatment. (— \blacktriangle) Group with the mean ribavirin dose <10.6 mg/kg/day. (--- \blacksquare) Group with the mean ribavirin dose ≥ 10.6 mg/kg/day. The Peg-IFN α -2b dose was dose-dependently correlated with c-EVR in both groups ($P < 0.0001$). There was no significant difference between the two ribavirin-dose groups ($P = 0.19$).

c-EVR rates according to Peg-IFN α -2b drug exposure using a percentage cut off and mean dose cut off

Table 5 shows the c-EVR rates according to the category of Peg-IFN α -2b doses during the first 12 weeks based on the

Table 5 The c-EVR rate according to Peg-IFN dose during weeks 0–12 based on the percentage of the planned dose and the mean doses

Peg-IFN α -2b dose (μ g/kg/week)	$\geq 80\%$	60–80%	<60%	Total
≥ 1.2	55%* (371/679)	71%** (12/17)	–	55% (383/696)
<1.2	32% (6/19)	38% (35/92)	22% (21/96)	30% (62/207)
Total	54% (377/698)	43% (47/109)	21% (21/96)	49% (445/903)

c-EVR, complete early virologic response; Peg-IFN, pegylated interferon.

* $P < 0.05$; patients with ≥ 1.2 μ g/kg/week vs <1.2 μ g/kg/week among the patients with more than 80% of the total prescribed dose of Peg-IFN α -2b. ** $P = 0.01$; patients with ≥ 1.2 μ g/kg/week vs <1.2 μ g/kg/week among the patients with more than 60–80% of the total prescribed dose of Peg-IFN α -2b.

percentage of the total prescribed dose and the mean doses. The whole c-EVR rate was 54% (377/698) for patients who received more than 80% of the prescribed dose, and 43% (47/109) in patients given 60–80% of the prescribed dose, and 21% (21/96) in patients given <60% of the prescribed dose of Peg-IFN α -2b. Among patients given $\geq 80\%$ of the prescribed dose of Peg-IFN α -2b, the c-EVR rate was significantly lower in patients given <1.2 μ g/kg/week of Peg-IFN α -2b than those given ≥ 1.2 μ g/kg/week (32% vs 55%, $P < 0.05$). On the other hand, even in patients given 60–80% of the prescribed dose of Peg-IFN α -2b, if they were given ≥ 1.2 μ g/kg/week of Peg-IFN α -2b, a higher c-EVR rate was attained in comparison with those given <1.2 μ g/kg/week (71% vs 38%, $P = 0.01$); the c-EVR rate in patients given 60–80% of the prescribed dose and ≥ 1.2 μ g/kg/week of Peg-IFN α -2b was not inferior to that in patients given $\geq 80\%$ of the prescribed dose and ≥ 1.2 μ g/kg/week of Peg-IFN α -2b.

Impact of dose exposure of Peg-IFN α -2b during the first 12 weeks of the treatment on HCV RNA negativity at week 24 and SVR

Patients positive for HCV RNA at week 24 week during Peg-IFN α -2b and ribavirin treatment were regarded as non-responders and stopped treatment [11]. We analysed the

relationship between the dose exposure to Peg-IFN α -2b during the first 12 weeks and HCV RNA negative rates at week 24 or SVR in 903 patients completing 12 weeks of treatment. As a result, HCV RNA negative rates at week 24 and SVR rates declined according to the decrease in the dose of Peg-IFN α -2b during the 12 weeks of treatment; patients given ≥ 1.5 , 1.2–1.5, 0.9–1.2 and < 0.9 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN α -2b during the first 12 weeks of the treatment showed HCV RNA negativity of 63%, 66%, 48% and 39%, respectively ($P < 0.0001$), and SVR of 46%, 43%, 30% and 20%, respectively ($P < 0.0001$).

DISCUSSION

Adherence to ribavirin was reported to be the important factor for EVR as well as that to Peg-IFN in most previous studies [2,11,12]. However, the drug exposure of Peg-IFN α -2b and ribavirin had not been analysed independently with respect to their individual influence on the anti-viral effect in these studies. Adherence to both drugs may be related factors, i.e. most patients who can tolerate a high dose of Peg-IFN are in good condition and thus can also receive a high dose of ribavirin. In the present study, the impact of the dose of Peg-IFN α -2b and ribavirin on the anti-viral effect was evaluated by multivariate logistic regression analysis, using the mean administration doses of both drugs during the first 12 weeks and baseline factors. As a result, the dose exposure of Peg-IFN α -2b was found to be the significant factor affecting c-EVR as well as baseline factors such as age, neutrophils and Plt values, but not ribavirin. This suggests that the c-EVR rate can be raised by maintaining the dose of Peg-IFN α -2b during the first 12 weeks in patients with disadvantageous factors at baseline. In fact, the c-EVR rate was higher in those who received ≥ 1.2 $\mu\text{g}/\text{kg}$ of Peg-IFN α -2b than in those given < 1.2 $\mu\text{g}/\text{kg}$ of Peg-IFN α -2b for aged patients over 60 years of age (≥ 1.2 $\mu\text{g}/\text{kg}$; 46% vs < 1.2 $\mu\text{g}/\text{kg}$; 28%, $P < 0.01$) or for patients with a low Plt value ($< 12 \times 10^4/\text{mm}^3$) (≥ 1.2 $\mu\text{g}/\text{kg}$; 45% vs < 1.2 $\mu\text{g}/\text{kg}$; 22%, $P < 0.001$). Therefore, a marked dose reduction of Peg-IFN α -2b should not be risked at the start even for aged patients or patients with lower Plt value, which is indicative of advanced fibrosis. The administration of ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN α -2b is desirable as a starting dose for achieving c-EVR even in these patients: that of < 1.2 $\mu\text{g}/\text{kg}/\text{week}$ can lead to a non-viral response or a late viral response. Independent evaluation of the c-EVR rate according to the degree of the ribavirin dose showed a stepwise decline as the total cumulative dose of Peg-IFN α -2b decreased. Therefore, the dose of Peg-IFN α -2b should be maintained as high as possible even in patients who have to reduce Peg-IFN α -2b to < 1.2 $\mu\text{g}/\text{kg}/\text{week}$. Using G-CSF for patients who develop severe neutropenia and are forced to decrease Peg-IFN can be beneficial, especially in the first 12 weeks.

The goal of 80% of the planned drug dosage for 80% of the assigned duration was derived from an adherence criterion

that had been adopted previously for assessment of the efficacy of other pharmaceutical agents, such as drugs to treat cancer and human immunodeficiency virus [16]. However, in Peg-IFN plus ribavirin therapy for patients with CH-C, the planned administration dose [17,18] differs on a body weight basis by 27% for Peg-IFN α -2b and 40% for ribavirin among patients of 50–100 kg of body weight, which would be equivalent to the same rate differences for 80% of the planned drug dosage. In detail, the target dose of Peg-IFN α -2b scheduled to be administered is 1.5 $\mu\text{g}/\text{kg}$, but the usual dose for the individual patient is from 1.28 to 1.76 $\mu\text{g}/\text{kg}/\text{week}$ based on body weight among patients weighing 50–100 kg according to the practice guidelines of the American Association for the Study of Liver Diseases and the manufacturer's drug information in the USA and Europe [17,18]. The range of ribavirin dose per kg of body weight is from 12 to 20 mg/kg/day. Therefore, in this study, the drug exposure was assessed from the average dose per kg of body weight.

In the evaluation of c-EVR rates according to Peg-IFN α -2b drug exposure using a percentage cut off and mean dose cut off in this study, the c-EVR rate of patients given < 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN α -2b was low (32%) even in those who received $\geq 80\%$ of the total planned doses of Peg-IFN α -2b. If given ≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN α -2b, the c-EVR rate (71%) in patients who received 60–80% of the total doses was not inferior to that in patients given $\geq 80\%$ of the total dose of Peg-IFN α -2b (54%). This means that patients whose starting dose of Peg-IFN α -2b is < 1.5 $\mu\text{g}/\text{kg}/\text{week}$ should not have their dosage reduced to 80% of the planned dose (< 1.2 $\mu\text{g}/\text{kg}/\text{week}$) in order to have a higher probability of c-EVR, while those given ≥ 1.5 $\mu\text{g}/\text{kg}/\text{week}$ of Peg-IFN α -2b at the start can have their dosage reduced to 80% (≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$) without lowering the c-EVR rate. Thus, the drug dose on a body weight basis itself should be examined as an index of the drug exposure in order to evaluate the anti-viral effect of both drugs accurately for patients with CH-C.

As for the impact of the drug exposure to ribavirin on c-EVR, the drug dose of ribavirin during the first 12 weeks was shown to have no relationship with the c-EVR rate, although it was precisely evaluated in this study, using doses actually taken on body weight. However, ribavirin can be more effective for decreasing the viral relapse after interferon or Peg-IFN α -2b and ribavirin combination therapy in patients with CH-C genotype 1 [2,3,19–24]. Recently, Shiffman *et al.* [15] have reported that a higher starting dose of ribavirin (1000–1600 mg/day) plus a regular dose of Peg-IFN α -2b with epoetin was associated with a lower relapse rate in treatment with CH-C genotype 1. Considering the viral relapse after treatment, it is thought that the ribavirin dose should not be reduced quickly in patients with mild side effects, even though it does not affect c-EVR. In fact, among the patients who attained c-EVR, a higher rate of viral relapse was found in the patients given < 10 mg/kg/day of the mean ribavirin dose during 48 weeks in comparison

with those given ≥ 10 mg/kg/day of the mean ribavirin dose in this study [26.9% (49/182) vs 12.4% (26/209), $P < 0.001$] (data not shown). It seems possible to start ribavirin at a lower dose and increase it by degrees with monitoring of Hb level during treatment of patients with mild anaemia or ischemic heart disease, because the ribavirin dose appears to affect the viral relapse as the total dose over 48 weeks, not during the first 12 weeks.

In conclusion, our results have demonstrated that Peg-IFN α -2b is dose-dependently correlated with c-EVR and maintaining as high a drug dose of Peg-IFN α -2b as possible (≥ 1.2 $\mu\text{g}/\text{kg}/\text{week}$) during the first 12 weeks can yield higher c-EVR rates, leading to better treatment outcomes for patients with CH-C genotype 1.

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Lamivudine-to-entecavir switching treatment in type B chronic hepatitis patients without evidence of lamivudine resistance

Nao Kurashige · Kazuyoshi Ohkawa · Naoki Hiramatsu · Takayuki Yakushijin · Kiyoshi Mochizuki · Tsugiko Oze · Shinichi Kiso · Tatsuya Kanto · Tetsuo Takehara · Akinori Kasahara · Yoshinori Doi · Akira Yamada · Kazuto Fukuda · Masahide Oshita · Eiji Mita · Hiroyuki Fukui · Toshihiko Nagase · Harumasa Yoshihara · Yasuharu Imai · Michio Kato · Takeshi Kashihara · Norio Hayashi

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Abstract

Purpose A considerable number of chronic hepatitis B (CH-B) patients remain under continuous lamivudine treatment, although switching treatment to entecavir could be beneficial. We investigated the antiviral efficacy of switching treatment to entecavir in CH-B patients without apparent evidence of lamivudine resistance during the preceding lamivudine treatment.

Methods Forty-four CH-B patients, who underwent lamivudine treatment for more than 6 months and showed no evidence of lamivudine resistance, switched to entecavir. Serial changes in hepatitis B virus (HBV) DNA were correlated with the patients' baseline HBV DNA at the commencement of entecavir administration. The entecavir-resistant substitution was examined by PCR-direct

sequencing. The median follow-up period of entecavir treatment was 20 (10–23) months.

Results All 31 patients with baseline HBV DNA <2.6 logcopies/ml maintained HBV DNA-negative status during entecavir treatment. Of seven patients having HBV DNA of 2.6–<4.0 logcopies/ml, all achieved undetectable HBV DNA at the end of follow-up. As for six patients having HBV DNA \geq 4.0 logcopies/ml, three patients achieved undetectable HBV DNA, whereas virological breakthrough was observed in one patient at month 15. An entecavir-resistant virus having rtM204V, rL180M and rS202G substitutions was detected in this patient.

Conclusions The lamivudine-to-entecavir switching treatment may be generally recommendable in CH-B patients without evidence of lamivudine resistance during

N. Kurashige · K. Ohkawa · N. Hiramatsu · T. Yakushijin · K. Mochizuki · T. Oze · S. Kiso · T. Kanto · T. Takehara · A. Kasahara · N. Hayashi (✉)
Department of Gastroenterology and Hepatology,
Osaka University Graduate School of Medicine,
Suita 565-0871, Japan
e-mail: hayashin@gh.med.osaka-u.ac.jp

Y. Doi
Otemae Hospital, Osaka, Japan

A. Yamada
Sumitomo Hospital, Osaka, Japan

K. Fukuda · Y. Imai
Ikeda Municipal Hospital, Ikeda, Japan

M. Oshita
Osaka Police Hospital, Osaka, Japan

E. Mita
National Hospital Organization Osaka National Hospital,
Osaka, Japan

H. Fukui
Yao Municipal Hospital, Yao, Japan

T. Nagase
Suita Municipal Hospital, Suita, Japan

H. Yoshihara
Osaka Rousai Hospital, Sakai, Japan

M. Kato
National Hospital Organization Minamiwakayama Medical
Center, Tanabe, Japan

T. Kashihara
Itami City Hospital, Itami, Japan

the preceding lamivudine treatment. However, great care should be taken with respect to the emergence of entecavir-resistance, especially in patients who do not respond well to the preceding lamivudine treatment.

Keywords Chronic hepatitis B · Lamivudine resistance · Entecavir-resistance

Introduction

Nucleos(t)ide analogs have been accepted as useful agents for suppressing hepatitis B virus (HBV) replication and disease progression in patients with type B chronic hepatitis (CH-B). Lamivudine, the first approved nucleoside analog, has been shown to provide short-term benefit for CH-B patients with respect to the reduction of HBV DNA, normalization of alanine aminotransferase (ALT) and improvement of liver histology [1, 2]. However, a serious shortcoming of lamivudine is the high incidence of drug resistance during long-term treatment. The detection rate of lamivudine resistance has been reported to be 24% at 1 year and 70% at 4 years of treatment [3]. Lamivudine resistance is caused by an rtM204V/I substitution within the reverse transcriptase domain of HBV polymerase gene [4–6]. An rtL180M substitution frequently emerges as a “replication-compensatory” one with the “resistance-causative” rtM204V/I substitution [4–7]. The emergence of lamivudine-resistant mutant HBV leads to the elevation of HBV DNA (“virological breakthrough”) and the subsequent increase of ALT (“breakthrough hepatitis”), resulting in disease progression. Adefovir dipivoxil and tenofovir disoproxil fumarate have been shown to be effective in both nucleos(t)ide analog-naïve and lamivudine-resistant CH-B patients [8–13].

Recently, entecavir has been demonstrated to exert antiviral efficacy in both nucleos(t)ide analog-naïve and lamivudine-refractory CH-B patients [14–16]. The frequency of entecavir-resistance has been reported to be less than 1% at 4 years of treatment in nucleos(t)ide analog-naïve CH-B patients [17]. On the other hand, in switching treatment to entecavir for lamivudine-refractory CH-B patients, most of whom developed lamivudine resistance during the preceding lamivudine therapy, the cumulative probability of entecavir-resistance has been reported to be no less than 40% at 4 years of treatment [17]. Entecavir-resistance has been shown to be established by amino acid substitution(s) at rt184, rt202 and/or rt250 along with the lamivudine-resistant rtM204V and rtL180M substitutions [18]. In the case of nucleos(t)ide analog-naïve patients, the requirement of at least three amino acid substitutions serves as a high genetic barrier to entecavir-resistance. By contrast, in the case of lamivudine-resistant patients, a

lower genetic barrier results in higher incidence of entecavir-resistance because two amino acid substitutions, rtM204V and rtL180M, already exist from the preceding lamivudine treatment. The reduced susceptibility to entecavir of the lamivudine-resistant virus compared with the wild-type virus is also a reason for the higher emergence rate of entecavir-resistance in lamivudine-resistant patients than in nucleos(t)ide analog-naïve ones [19].

Although lamivudine is not currently recommended as a first-line drug for nucleos(t)ide analog-naïve CH-B, a considerable number of CH-B patients are under continuous treatment with lamivudine. In these patients, the switch to entecavir treatment could be advantageous over continuation of lamivudine treatment by offering stronger antiviral efficacy and less chance of drug resistance. With respect to the manner of emergence of entecavir-resistance, switching a patient’s treatment may be more appropriate before the appearance of lamivudine resistance than after its development. However, the usefulness of lamivudine-to-entecavir switching treatment has not been assessed in CH-B patients without apparent evidence of lamivudine resistance.

This led us to investigate the antiviral efficacy and emergence of entecavir-resistance in CH-B patients who showed no evidence of lamivudine resistance during the preceding lamivudine treatment and underwent the switching treatment to entecavir.

Patients and methods

Patients

This study included 44 consecutive CH-B patients from 10 institutions in the Osaka area of Japan (Otemae Hospital, Sumitomo Hospital, Osaka Police Hospital, Suita Municipal Hospital, Yao Municipal Hospital, Osaka Rousai Hospital, Ikeda Municipal Hospital, National Hospital Organization Osaka National Hospital, Itami City Hospital and Osaka University Hospital) who underwent continuous lamivudine treatment (100 mg/day) for more than 6 months and showed no apparent evidence of lamivudine resistance. Before starting the preceding lamivudine treatment, all patients had abnormal ALT, positive hepatitis B surface antigen (HBsAg) and a detectable level of HBV DNA according to PCR-based assay (Amplicor HB Monitor, Roche Diagnostics) or branched DNA assay (Quantiplex HBV DNA, Chiron). None of them showed evidence of dual infection with hepatitis C virus or human immunodeficiency virus, or other forms of liver diseases such as alcoholic liver disorder, autoimmune hepatitis and drug-induced liver injury. The total duration of the preceding lamivudine treatment ranged from 6 to 73 (median, 14)

months. The absence of lamivudine resistance was defined by no detection of the rtM204V/I substitution as measured by the PCR–enzyme linked minisequence assay (ELMA) (Sumitomo Metal Industries) [20] for 33 patients, or by the lack of virological breakthrough as judged by more than 1 log increment in HBV DNA from the nadir for the remaining 11 patients. All of the 44 patients switched to 0.5 mg/day of entecavir administration. After the beginning of entecavir treatment, liver function tests and HBV markers were measured at 1- to 2-month intervals. When virological breakthrough was observed during follow-up, entecavir-resistance-associated mutations were examined by means of a PCR-direct sequencing method. The follow-up period of entecavir treatment ranged from 10 to 23 (median 20) months.

Baseline characteristics of the patients

At the commencement of switching treatment to entecavir, the 28 males and 16 females were aged 33–79 (median 59) years. Seventeen patients (39%) tested positive for hepatitis B e antigen (HBeAg), and antibody against HBeAg (anti-HBe) developed in all of the 27 HBeAg-negative patients. Among the 27 HBeAg-negative patients, four achieved HBeAg clearance during the preceding lamivudine treatment. HBV DNA at baseline varied among patients from <2.6 to 5.2 logcopies/ml. The baseline ALT ranged from 11 to 78 (median 25) IU/l. Regarding the liver diseases of the patients, 27 (61%) showed features of chronic hepatitis, 11 (25%) of liver cirrhosis and six (14%) of hepatocellular carcinoma (HCC) according to liver biopsy and/or abdominal imaging procedures. HBV genotype was examined for 14 patients, and all of them had HBV genotype C, the most predominant genotype in Japan. Informed consent was obtained from all patients.

Serological and virological markers of HBV

HBsAg, HBeAg and anti-HBe were determined by chemiluminescent immunoassay. HBV DNA was measured by the PCR-based method (Amplicor HBV monitor, Roche Diagnostics) whose lower detection limit is 2.6 logcopies/ml. Lamivudine-resistant rtM204V/I substitution was examined by the PCR–ELMA method (Sumitomo Metal Industries) (20), which is capable of detecting the mutant virus in a mixed viral population if it is present at more than 10% of the total population. The entecavir-resistance-associated substitutions and HBV genotype were determined by a PCR-direct sequencing method. As for oligonucleotide primers for PCR reaction, the outer primer sets were BF5 (5'-AAG AGA CAG TCA TCC TCA GG-3', nt 3183–3202) and BR1s (5'-AAA AAG TTG CAT GGT GCT GG-3', nt 1825–1806), and the inner primer sets were

BF6 (5'-CCT CCA ATT TGT CCT GGC TA-3', nt 350–369) and BR8 (5'-TTG CGT CAG CAA ACA CTT GG-3', nt 1195–1176). After DNA extraction, the DNA sample was subjected to the PCR reaction for 35 cycles (denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min) using the inner primer set, followed by a final extension at 72°C for 10 min. If amplification was not successful by the single PCR reaction, the nested PCR was conducted; the first round PCR was done using the outer primer sets for 35 cycles, and the aliquot of the product was used for the second round PCR for 30 cycles using inner primer sets. All sequencing reactions of the PCR products were carried out using the BigDye Terminator Ver. 3.1 Cycle Sequencing Kit, and 3100 or 3730 Genetic Analyzer (Applied Biosystems), which allowed determination of the amino acid sequences of nt85–344. For determining the HBV genotype, nucleotide sequences obtained in each of the patients were aligned along with representative HBV strains of genotype A–H, and a phylogenetic tree was constructed in the homepage of DNA Data Bank of Japan (<http://www.ddbj.nig.ac.jp>).

Statistical analysis

Statistical analysis for group comparison was performed by Fisher's exact probability test and Mann–Whitney's non-parametric *U* test using the SPSS version 15.0J software (SPSS Inc, Chicago, IL). A *p* value of less than <.05 was considered to be significant.

Results

Classification of patients who underwent lamivudine-to-entecavir switching treatment according to baseline HBV DNA

The 44 CH-B patients who underwent the switching treatment from lamivudine to entecavir were first classified according to their baseline HBV DNA at the commencement of entecavir administration. HBV DNA was not detectable (<2.6 logcopies/ml) in 31 patients (70%) at baseline. Seven patients (16%) had baseline HBV DNA of 2.6–<4.0 logcopies/ml. In the remaining six patients (14%), the baseline HBV DNA was ≥ 4.0 logcopies/ml. When patient clinical characteristics were compared among the three patient groups (Table 1), nine (29%) of the 31 patients with baseline HBV DNA <2.6 copies/ml tested positive for HBeAg at the commencement of switching treatment to entecavir, compared with five of the six (83%) patients with baseline HBV DNA ≥ 4.0 copies/ml (*p* < .05). Gender ratio, age, ALT at baseline, liver disease, duration of the preceding lamivudine treatment and

Table 1 Patient clinical characteristics and the therapeutic efficacy in 44 CH-B patients in relation to their baseline HBV DNA

	Baseline HBV DNA		
	<2.6 logcopies/ml (n = 31)	2.6–<4.0 logcopies/ml (n = 7)	≥4.0 logcopies/ml (n = 6)
At the commencement of switching treatment to entecavir			
Gender (male/female)	19/12	5/2	4/2
Age (years)	60 (35–79) ^a	65 (41–69)	55 (33–65)
HBeAg (positive/negative)	9/22	3/4	5/1 ^b
HBV DNA (logcopies/ml)	<2.6	3.1 (2.6–3.6) ^c	4.6 (4.0–5.2) ^{c,d}
rtM204V/I mutation (absence/NT)	23/8	5/2	5/1
ALT (IU/l)	25 (11–64)	31 (13–46)	20 (17–78)
Chronic hepatitis/cirrhosis/HCC	19/7/5	4/2/1	4/2/0
Follow-up period of entecavir treatment (months)	19 (10–23)	19 (10–22)	20 (16–22)
The rate of undetectable HBV DNA level during follow-up	31 (100%)	7 (100%)	3 (50%) ^c
Emergence of entecavir-resistance during follow-up	0 (0%)	0 (0%)	1 (17%)
At the commencement of preceding lamivudine treatment			
HBeAg (positive/negative)	12/19	4/3	5/1
HBV DNA (logcopies/ml)	6.5 (4.3–7.6)	6.6 (6.2–7.6)	7.6 (5.9–7.6)
Duration of preceding lamivudine treatment (months)	15 (6–73)	10 (7–42)	9 (8–32)

NT not tested

^a Values are expressed as median (range)

^b $p < .05$ versus baseline HBV DNA <2.6 logcopies/ml group

^c $p < .01$ versus baseline HBV DNA <2.6 logcopies/ml group

^d $p < .01$ versus baseline HBV DNA of 2.6–<4.0 logcopies/ml group

follow-up period of entecavir treatment did not differ among the three groups. Also, there was no significant difference in HBV DNA and the frequency of positive HBeAg at the commencement of preceding lamivudine treatment among them.

Antiviral efficacy and drug resistance in lamivudine-to-entecavir switching treatment in relation to baseline HBV DNA

Next, we investigated serial changes in HBV DNA after the switch from lamivudine to entecavir treatment in CH-B patients in relation to the baseline HBV DNA. All 31 patients with baseline HBV DNA <2.6 logcopies/ml maintained undetectable HBV DNA during the follow-up period of entecavir treatment. Figure 1 shows the longitudinal evaluation of HBV DNA during the switching treatment to entecavir in patients with a detectable level of baseline HBV DNA. In patients having baseline HBV DNA of 2.6–<4.0 logcopies/ml (Fig. 1a), all of the seven patients achieved sustained undetectable HBV DNA during follow-up, although HBV DNA was transiently detected in one patient. As for patients having baseline HBV DNA ≥4.0 logcopies/ml (Fig. 1b), three (50%) of the six patients achieved sustained undetectable HBV DNA during follow-up. In two patients, HBV DNA was not cleared

entirely, but declined to 2.9 and 2.7 logcopies/ml at month 18, respectively. In sequencing analysis at that time, the former patient had the lamivudine-resistant rtM204I substitution, although it was not detected by the PCR–ELMA assay at the start of entecavir treatment. The latter patient had no drug resistance-associated substitutions. In the sixth patient, HBV DNA decreased initially, but virological breakthrough was seen at month 15. The entecavir-resistant virus was detected after virological breakthrough. The detailed disease course of the entecavir-resistant patient is described below. As for the relationship of baseline HBV DNA to the frequency of undetectable HBV DNA, HBV DNA was cleared more frequently in patients with baseline HBV DNA <2.6 logcopies/ml than in those with baseline HBV DNA ≥4.0 logcopies/ml (100 vs. 50%, $p < .01$) (Table 1).

Serial changes in ALT during lamivudine-to-entecavir switching treatment were further examined. Among the 31 patients with baseline HBV DNA <2.6 logcopies/ml, the baseline ALT was within the normal range (≤40 IU/l) in 27 patients, 24 of whom showed sustained ALT normalization during follow-up. In the remaining three patients, ALT became slightly abnormal (≤60 IU/l) during follow-up. As for four patients with abnormal baseline ALT, the level was normalized in three, whereas a slight elevation of ALT (≤60 IU/l) continued in one during follow-up.

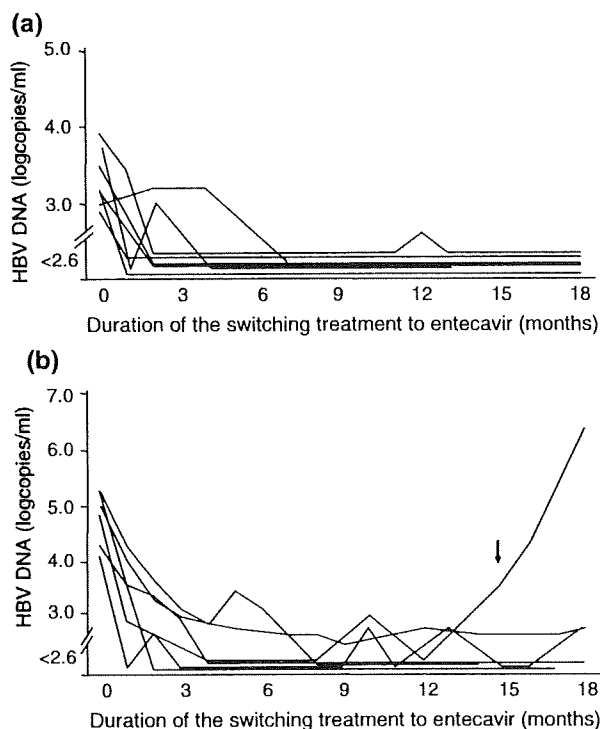


Fig. 1 Changes in HBV DNA after commencement of switching treatment from lamivudine to entecavir in CH-B patients with baseline HBV of (a) 2.6–<4.0 logcopies/ml and (b) \geq 4.0 logcopies/ml. The black arrow indicates the time point of virological breakthrough

Among the 13 patients having a detectable level of baseline HBV DNA, five patients (three with baseline HBV DNA of 2.6–<4.0 logcopies/ml and two with baseline HBV DNA \geq 4.0 logcopies/ml) had abnormal ALT at baseline but showed ALT normalization during follow-up. In the remaining eight patients, ALT continued to be normal from the beginning of entecavir treatment.

Disease course of the CH-B patients showing entecavir-resistance during lamivudine-to-entecavir switching treatment

The disease course of the entecavir-resistant patient is shown in Fig. 2. This patient was a 33-year-old HBeAg-positive male, whose liver biopsy showed features of chronic hepatitis. He underwent the preceding lamivudine treatment for 8 months. HBV DNA decreased from >7.6 to 4.6 logcopies/ml, and ALT was normalized during the lamivudine therapy. The rtM204V/I substitution was not detected before the switch to entecavir treatment by the PCR–ELMA analysis. After the commencement of entecavir treatment, HBV DNA was cleared at month 5. However, virological breakthrough was seen at month 15, and HBV DNA was further increased to 6.1 logcopies/ml

at month 18. The sequencing analysis at month 18 revealed the rtM204V, rtL180M and rtS202G substitutions. Two additional substitutions, rtL267M and rtQ316H, were also found, when the amino acid sequences were compared with three representative genotype C HBV isolates (Genbank accession nos. V00867, X01587 and D00630) [21–23]. Breakthrough hepatitis was not evident after the emergence of entecavir-resistant mutant virus. The sequencing analysis also revealed that he was infected with HBV of genotype C.

Discussion

Entecavir treatment has been shown to exhibit more powerful antiviral efficacy and less frequent drug resistance than lamivudine treatment in nucleos(t)ide analog-naïve CH-B patients [14, 15, 17]. Entecavir is also effective in patients showing lamivudine resistance during the preceding lamivudine treatment, but its efficacy is limited due to the higher incidence of entecavir-resistance, compared with nucleos(t)ide analog-naïve ones [16, 17]. This is because entecavir-resistance is established based on two lamivudine-resistant substitutions, rtM204V and rtL180M, and additional mutation(s) occurring at rt184, rt202 and/or rt250 [18]. A considerable number of CH-B patients remain under continuous lamivudine treatment, while the lamivudine-to-entecavir switching treatment could yield a practical benefit. The switching treatment may be more promising for patients before the appearance of lamivudine resistance than after its development. In the present study, we investigated the efficacy of lamivudine-to-entecavir switching treatment in CH-B patients without apparent evidence of lamivudine resistance during the preceding lamivudine treatment.

We evaluated the antiviral efficacy of the switching treatment to entecavir in relation to the baseline HBV DNA at the commencement of the entecavir administration. In all patients having baseline HBV DNA <2.6 logcopies/ml, who revealed a good response to the preceding lamivudine treatment, HBV DNA continued to be undetectable during the switching treatment to entecavir. Also, all patients having baseline HBV DNA of 2.6–<4.0 logcopies/ml achieved sustained undetectable HBV DNA during the follow-up period of entecavir treatment. Among six patients having baseline HBV DNA \geq 4.0 logcopies/ml, who did not respond well to the preceding lamivudine treatment, HBV DNA was cleared in three during follow-up. Its reduction by up to 3.0 logcopies/ml was seen in two additional cases without emergence of the entecavir-resistant virus. Thus, the antiviral efficacy of the lamivudine-to-entecavir switching treatment was exhibited in almost all CH-B patients in parallel with that of the preceding