

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 $\mu\text{g/kg/week}$ of Peg-IFN and that given < 1.4 $\mu\text{g/kg/week}$ (1.4 $\mu\text{g/kg/week}$ 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 $\mu\text{g/kg/week}$ and strongly suppress it to 7% (3/41) in the group given ≥ 1.4 $\mu\text{g/kg/week}$ of Peg-IFN.

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

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 negativitation.

[¶]METAVIR fibrosis score.

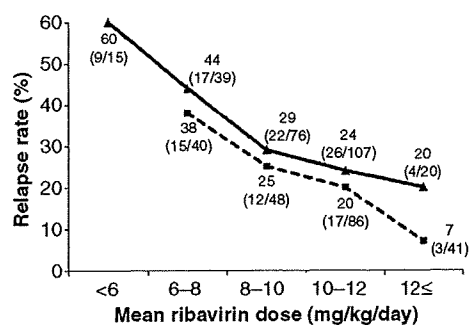


Fig. 2 Relapse rate according to Peg-IFN α -2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses. (— \blacktriangle) Group with the mean Peg-IFN dose <1.4 $\mu\text{g}/\text{kg}/\text{week}$; (--- \blacksquare) Group with the 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					
Peg-IFN dose (mean, µg/kg/week)		12–48 weeks			
		≥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					
Ribavirin dose (mean, mg/kg/day)		12–48 weeks			
		≥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

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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.

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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 ($\times 10^4/\text{mm}^3$)	442 ± 47
Haemoglobin (g/dL)	14.1 ± 1.4
Platelets ($\times 10^4/\text{mm}^3$)	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 $\mu\text{g}/\text{kg}$ based on body weight (body weight 35–45 kg, 60 μg ; 46–60 kg, 80 μg ; 61–75 kg, 100 μg ; 76–90 kg, 120 μg ; 91–120 kg, 150 μg) 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 $< 1500/\text{mm}^3$, the neutrophil count to $< 750/\text{mm}^3$ or the platelet (Plt) count to $< 8 \times 10^4/\text{mm}^3$, 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 univariate

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.

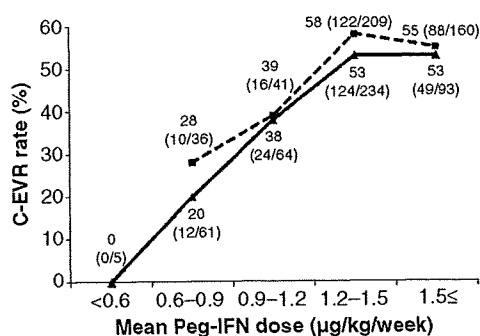


Fig. 1 Complete-EVR rate according to pegylated interferon alpha-2b (Peg-IFN α -2b) and ribavirin doses during weeks 0–12 for patients who completed 12 weeks of treatment. (—▲) Group with the mean ribavirin dose <10.6 mg/kg/day. (---■) Group with the mean ribavirin dose ≥ 10.6 mg/kg/day. The Peg-IFN 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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Factors contributing to antiviral effect of adefovir dipivoxil therapy added to ongoing lamivudine treatment in patients with lamivudine-resistant chronic hepatitis B

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Abstract

Purpose The antiviral effect of adefovir dipivoxil (ADV) added to ongoing lamivudine (LAM) treatment for LAM-resistant chronic hepatitis B (CHB) differs among patients. We investigated clinical factors affecting the response to ADV therapy in LAM-resistant CHB.

Methods The subjects were 75 LAM-resistant CHB patients treated with ADV in addition to LAM. Virological response (VR) was defined as HBV DNA clearance (<2.6 logcopies/ml) at 12 months after the start of ADV therapy. Clinical factors contributing to VR were examined by univariate and multivariate analyses.

Results Lower HBV DNA at baseline and negative hepatitis B e antigen (HBeAg) were significant factors affecting VR in univariate analysis. In multivariate analysis, lower HBV DNA at baseline ($P = 0.005$), negative HBeAg ($P = 0.009$), and higher ALT ($P = 0.036$) were significant independent factors contributing to VR. In HBeAg-positive patients, HBV DNA clearance was more frequently observed during ADV therapy in patients with baseline HBV DNA ≤ 7.0 logcopies/ml than in those with baseline HBV DNA > 7.0 logcopies/ml. By contrast, the link of lower HBV DNA at baseline to better therapeutic response was not evident in HBeAg-negative patients.

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Conclusion In ADV therapy added to ongoing LAM treatment for LAM-resistant CHB, lower baseline HBV DNA and negative HBeAg contributed to a better antiviral effect. Addition of ADV should be done promptly before marked increase in HBV DNA, especially in CHB patients showing LAM resistance positive for HBeAg.

Keywords Adefovir dipivoxil · Lamivudine resistance · Chronic hepatitis B

Introduction

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV) [1]. Chronic HBV infection can cause liver cirrhosis and hepatocellular carcinoma (HCC), resulting in hepatic disease-related deaths of 500,000 to 1.2 million persons [2, 3]. To prevent disease progression and improve the prognosis of patients with chronic HBV infection, HBV DNA replication must be continuously suppressed as much as possible by antiviral therapy. For this purpose, nucleos(t)ide analogs are currently used for a wide range of patients with chronic HBV infection because of their strong antiviral activities and fewer side effects.

Lamivudine (LAM) is the first approved nucleos(t)ide analog for chronic hepatitis B (CHB) patients, but the increasing incidence of LAM resistance during long-term LAM therapy is a serious problem. The emergence rate of the LAM-resistant virus has been reported to be 24% at 1 year and 70% at 4 years of treatment [4]. Almost all LAM resistance is caused by rtM204V/I mutation occurring in the reverse transcriptase domain of the HBV polymerase gene [5].

To counteract this resistance, adefovir dipivoxil (ADV) was considered as it exerts antiviral effects not only on nucleos(t)ide analog-naïve CHB patients but also on LAM-resistant ones [6–9]. ADV-resistant mutation has been reported to be detected in 11% of patients at 3 years and 29% at 5 years for nucleos(t)ide analog-naïve CHB patients [10]. ADV resistance results from rtA181V/T and/or rtN236T mutation [10]. Either switching from LAM to ADV or adding ADV to LAM has been shown to be effective for LAM-resistant CHB patients. In the case of switching from LAM to ADV, ADV resistance has been reported to appear in 18% of patients at 1 year, which is more frequent than in the case of ADV monotherapy for nucleos(t)ide analog-naïve patients [11]. On the other hand, in the case of ADV administration in addition to LAM, the emergence of resistant virus for both LAM and ADV has been reported to be rare for at least 3 years of treatment [12]. Therefore, ADV therapy added to ongoing LAM treatment is currently accepted as the main therapeutic

regimen for LAM-resistant CHB patients rather than a switch from LAM to ADV. However, the antiviral effect of ADV therapy in addition to LAM treatment differs among patients with LAM-resistant CHB.

In this study, we investigated clinical factors influencing the therapeutic efficacy of ADV therapy added to ongoing LAM treatment in LAM-resistant CHB patients.

Patients and methods

Patients

The participating centers were 12 institutions in the Osaka area of Japan (Otemae Hospital, Sumitomo Hospital, Osaka Police Hospital, NTT Nishinohon Osaka Hospital, Higashiosaka City General Hospital, Suita Municipal Hospital, Osaka Rousai Hospital, Kinki Central Hospital, Ikeda Municipal Hospital, National Hospital Organization Osaka National Hospital, Itami City Hospital, and Osaka University Hospital). The subjects were 75 consecutive CHB patients showing LAM resistance. Before the preceding LAM therapy, they all had had hepatitis B surface antigen (HBsAg) for more than 6 months and levels of HBV DNA detectable by the polymerase chain reaction (PCR) method [13]. None of them tested positive for hepatitis C virus antibody or human immunodeficiency virus antibody, nor was there evidence of other forms of liver diseases, such as alcoholic liver disease, drug-induced liver disease, or autoimmune hepatitis.

Anti-HBV treatment

All patients were administered 100 mg of LAM daily. Thirteen (17%) patients had had a history of interferon (IFN) therapy. LAM resistance was judged by detection of rtM204V/I mutation (for 37 patients) or by the existence of virological breakthrough (for 38 patients). Virological breakthrough was defined as the reappearance of detectable HBV DNA of more than 1 log increase in HBV DNA from the nadir on repeated occasions. The median duration of the preceding LAM therapy was 38 (range, 11–83) months. After the emergence of LAM resistance, all patients received 10 mg of ADV daily in addition to ongoing LAM therapy. After the commencement of ADV therapy, liver function and HBV DNA tests were conducted monthly for the first 6 months and every 2 months thereafter. Hepatitis B e antigen (HBeAg) and antibody to HBeAg (anti-HBe) were checked every 2 months. The median follow-up duration of ADV therapy was 22 (range 12–51) months. HBV DNA clearance (<2.6 logcopies/ml) at 12 months after the beginning of ADV therapy was defined as a virological response (VR).

Baseline characteristics of the patients

The baseline characteristics of the patients at the commencement of ADV therapy were as follows. They were 59 males and 16 females, with a median age of 54 (range 27–79) years. Forty-one (55%) tested positive for HBeAg, and anti-HBe developed in 34 patients. The virus was genotyped for 13 patients, all of whom were infected with HBV of genotype C. The HBV DNA ranged from 3.1 to >7.6 (median 7.1) logcopies/ml, and the median ALT level ranged from 15 to 500 (median 105) IU/L. The median levels of total bilirubin and albumin were 0.8 (range 0.4–3.9) mg/dl and 3.9 (range 2.1–4.8) g/dl, respectively. The median platelet counts were 11.7 (range 3.5–25.5) × 10⁴/mm³. Of the 75 patients, 27 (36%) showed features of cirrhosis by liver biopsy and/or imaging procedures. Five patients (7%) developed HCC as detected by imaging modalities.

HBV testings

HBsAg, HBeAg, and anti-HBe were examined by chemiluminescent immunoassay. HBV DNA was measured by the PCR-based method (Amplicor HBV monitor, Roche Diagnostics, Tokyo, Japan) [13], with a lower detection limit of 2.6 logcopies/ml. The LAM-resistant rtM204V/I mutation was examined by PCR-enzyme-linked minisequence assay [14]. HBV genotype was determined based on PCR-direct sequencing of portions of core and polymerase genes. The primers used for this study were BF1s (5'-TTT TTC ACC TCT GCC TAA TCA-3', nt 1821–1841), BR3 (5'-TTC CCG AGA TTG AGA TCT TC-3', nt 2440–2421), 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) [15, 16].

Statistical analysis

Group comparisons were carried out by the chi-square test, Student's *t* test and Mann–Whitney's *U* test. Independent

factors contributing to VR during ADV therapy added to ongoing LAM treatment were estimated using multivariate multiple logistic regression analysis in combination with stepwise regression analysis. A *P*-value of less than 0.05 (two-tailed) was considered to indicate a significant difference. All statistical analyses were performed using the SPSS version 15.0J software (SPSS, Chicago, IL).

Results

Virological and biochemical response to ADV therapy added to ongoing LAM in CHB patients showing LAM resistance

Of the 75 CHB patients showing LAM resistance who underwent ADV therapy added to ongoing LAM treatment, HBV DNA clearance was achieved in 29 (39%) of 75 at 6 months, 35 (47%) of 75 at 12 months, and 34 (72%) of 47 at 24 months. Among the HBeAg-positive patients, HBeAg loss was observed in 8 (20%) of 41 at 6 months, 7 (18%) of 39 at 12 months, and 6 (22%) of 27 at 24 months. As for the biochemical response, ALT normalization (≤40 IU/l) was seen in 57 (76%) of 75 at 6 months, 56 (75%) of 75 at 12 months, and 40 (85%) of 47 at 24 months of treatment.

Pretreatment clinical factors associated with therapeutic response to ADV in addition to LAM treatment

We first investigated pretreatment clinical factors associated with the therapeutic efficacy of ADV added to ongoing LAM treatment by univariate analysis. The baseline characteristics of patients at the beginning of ADV therapy in addition to LAM in the presence or absence of VR are shown in Table 1. Patients showing VR had significantly lower HBV DNA at baseline than patients who did not achieve VR [median 6.3 (range 3.1 to >7.6) vs. 7.3

Table 1 Patient clinical characteristics at the beginning of ADV therapy in addition to LAM in LAM-resistant CHB patients in the presence or absence of virological response (VR)

Clinical characteristics	VR (n = 35)	Non-VR (n = 40)	P value
Gender (male/female)	26/9	33/7	0.386
Age (years)	52 (28–67)	55 (27–79)	0.896
Duration of prior LAM therapy (months)	38 (12–83)	37 (13–64)	0.856
Positive HBeAg	12 (34%)	29 (73%)	0.001
HBV DNA (logcopies/ml)	6.3 (3.1 to >7.6)	7.3 (3.9 to >7.6)	0.002
ALT (IU/l)	106 (16–500)	75 (15–455)	0.136
Total bilirubin (mg/dl)	0.9 (0.4–3.9)	0.7 (0.4–3.9)	0.664
Albumin (g/dl)	4.0 (2.4–4.8)	3.8 (2.1–4.6)	0.351
Platelet count (×10 ⁴ /mm ³)	12.2 (4.8–24.1)	11.5 (3.5–25.5)	0.854
Liver disease (chronic hepatitis/cirrhosis)	20/15	28/12	0.247
Presence of HCC (%)	2 (6%)	3 (8%)	0.757

Continuous variables are expressed as median (range)

Table 2 Baseline factors affecting virological response (logistic regression analysis, stepwise method)

Factors	Category	Odds ratio	95% CI	P
Gender	Male/female			NS
Age (years)	By 1 year			NS
Duration of prior LAM therapy (months)	By 1 month			NS
HBeAg	Negative/positive	5.766	1.855–36.62	0.009
HBV DNA (logcopies/ml)	By 1 logcopy/ml	2.362	1.335–5.178	0.005
ALT (IU/l)	By 1 IU/l	1.006	1.000–1.011	0.036
Total bilirubin (mg/dl)	By 1 mg/dl			NS
Albumin (g/dl)	By 1 g/dl			NS
Platelet count ($\times 10^4/\text{mm}^3$)	By $1 \times 10^4/\text{mm}^3$			NS
Liver disease	Chronic hepatitis/cirrhosis			NS
Presence of HCC (%)	No/yes			NS

CI Confidence interval, NS not significant

(range 3.9 to >7.6), $P = 0.002$]. HBeAg was detected in only 12 (34%) of 35 patients with VR, compared with 29 (73%) of 40 patients without VR ($P = 0.001$). Gender ratio, age, duration of preceding LAM therapy, ALT, total bilirubin, albumin, platelet counts, disease severity, and presence of HCC did not differ between VR and non-VR patients.

Factors affecting the therapeutic response to ADV therapy in addition to ongoing LAM were also evaluated by multivariate analysis (Table 2). Eleven pretreatment clinical factors were applied to the analysis as variables. Two factors, lower baseline HBV DNA ($P = 0.005$, odds ratio: 2.362, 95% confidence interval: 1.335–5.178) and negative HBeAg ($P = 0.009$, odds ratio: 5.766, 95% confidence interval: 1.855–36.62), were selected as significant independent factors affecting VR, as was the case for univariate analysis. In addition, higher baseline ALT was also chosen as a significant independent factor ($P = 0.036$, odds ratio 1.006, 95% confidence interval: 1.000–1.011). As for the biochemical response to ADV therapy added to LAM, no pretreatment clinical factors showed a significant relationship with the occurrence of ALT normalization in our 75 LAM-resistant CHB patients.

HBV DNA clearance during ADV therapy in addition to ongoing LAM treatment according to HBeAg status

Next, we investigated HBV DNA clearance during ADV therapy added to ongoing LAM treatment in LAM-resistant CHB patients positive or negative for HBeAg (Fig. 1). In HBeAg-positive patients, HBV DNA was cleared in 8 (20%) of 41 at 6 months, 12 (29%) of 41 at 12 months, and 16 (59%) of 27 at 24 months. On the other hand, HBV DNA clearance was seen in 21 (62%) of 34 at 6 months, 23 (68%) of 34 at 12 months, and 18 (90%) of 20 at 24 months in HBeAg-negative patients. A significant difference ($P < 0.05$) in the frequency of HBV DNA clearance was

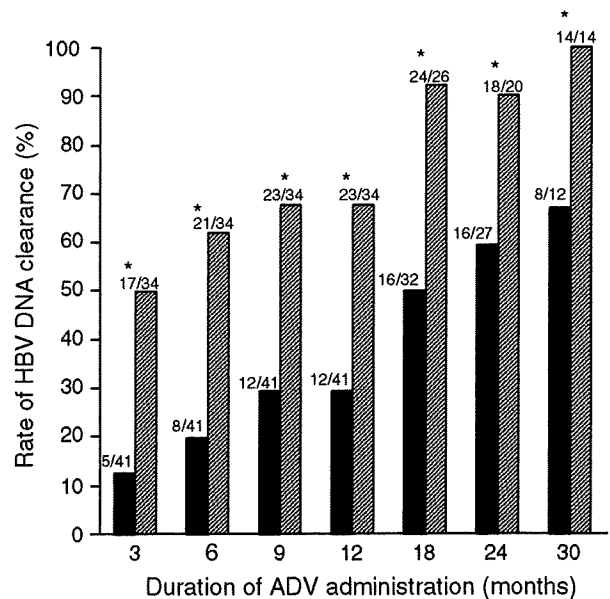


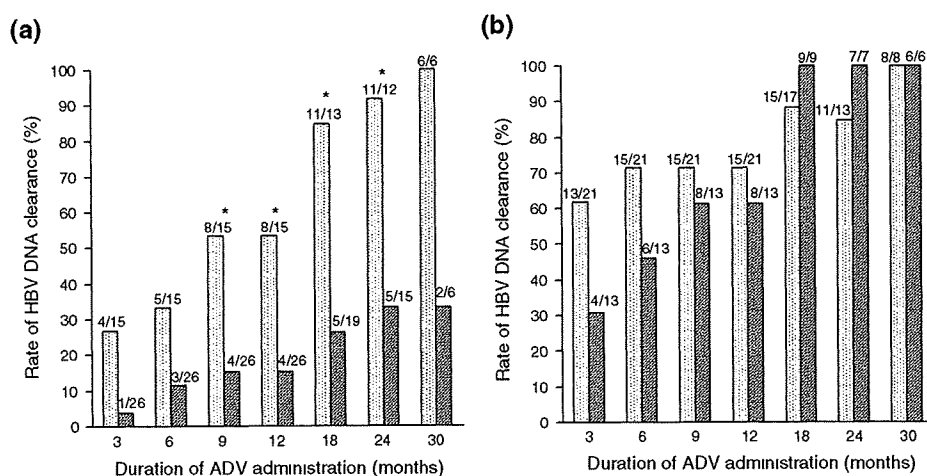
Fig. 1 Rates of HBV DNA clearance in CHB patients positive or negative for HBeAg during ADV therapy in addition to LAM. * $P < 0.05$ between HBeAg-positive and HBeAg-negative patients. Solid bars HBeAg-positive patients, hatched bars HBeAg-negative patients

observed between HBeAg-positive and HBeAg-negative patients at 3, 6, 9, 12, 18, 24, and 30 months of treatment. Thus, patients negative for HBeAg tended to respond to ADV therapy added to ongoing LAM treatment better than those positive for it in LAM-resistant CHB.

HBV DNA clearance during ADV therapy in addition to ongoing LAM treatment in relation to HBeAg status and baseline HBV DNA

We examined HBV DNA clearance during ADV therapy in addition to ongoing LAM treatment in HBeAg-positive and

Fig. 2 Rates of HBV DNA clearance during ADV therapy in addition to LAM according to HBV DNA at baseline in **a** HBeAg-positive CHB patients and **b** HBeAg-negative CHB patients. * $P < 0.05$ between patients with low (≤ 7.0 logcopies/ml) and high (> 7.0 logcopies/ml) HBV DNA. *Dotted bars* Patients with HBV DNA ≤ 7.0 logcopies/ml at baseline, *hatched bars* patients with HBV DNA > 7.0 logcopies/ml at baseline



HBeAg-negative CHB patients in relation to baseline HBV DNA. In the case of HBeAg-positive CHB patients (Fig. 2a), the rates of HBV DNA clearance were 33% (5/15) at 6 months, 53% (8/15) at 12 months, and 92% (11/12) at 24 months in patients with low viremia (baseline HBV DNA ≤ 7.0 logcopies/ml). By contrast, the frequencies of HBV DNA clearance were only 12% (3/26) at 6 months, 15% (4/26) at 12 months, and 33% (5/15) at 24 months in patients with high viremia (baseline HBV DNA > 7.0 logcopies/ml). A significant difference ($P < 0.05$) in the frequency of HBV DNA clearance was observed between patients with low and high viremia at 9, 12, 18, and 24 months of treatment. In the case of HBeAg-negative patients (Fig. 2b), the rates of HBV DNA clearance were 71% (15/21) at 6 months, 71% (15/21) at 12 months, and 85% (11/13) at 24 months in patients with low viremia (baseline HBV DNA ≤ 7.0 logcopies/ml). The frequencies of HBV DNA clearance were 46% (6/13) at 6 months, 62% (8/13) at 12 months, and 100% (7/7) at 24 months in patients with high viremia (baseline HBV DNA > 7.0 logcopies/ml). No significant differences were observed in the frequency of HBV DNA clearance between patients with low and high viremia. According to these findings, the relevance of lower baseline HBV DNA for achieving a better antiviral effect was evident only in HBeAg-positive patients, but not in HBeAg-negative ones in ADV therapy added to LAM treatment for LAM-resistant CHB.

Discussion

This study investigated factors affecting the antiviral efficacy of ADV therapy added to ongoing LAM treatment in LAM-resistant CHB patients. Therapeutic efficacy was assessed as the presence or absence of VR. Both univariate and multivariate analyses revealed that lower baseline

HBV DNA and negative HBeAg were strong factors associated with a better therapeutic response. Another significant factor revealed by multivariate analysis was high ALT, although it was weaker than the other two factors. In previous investigations, female gender, lower baseline HBV DNA, negative HBeAg, higher ALT, and genotype D rather than A have been reported to contribute to better VRs to ADV therapy in nucleos(t)ide-naïve and LAM-resistant CHB patients [17–21]. Our results agreed partially with them. The present study, as well as previous studies [18, 19], also revealed that a high baseline ALT may be a determining factor for a better response to ADV therapy in addition to LAM treatment in LAM-resistant CHB. This may be because the host immune response against viral antigens induced by active breakthrough hepatitis has a favorable antiviral effect during ADV therapy. In this study, however, a low baseline viremia level was shown to be a stronger factor than high baseline ALT. The baseline ALT level was the third factor contributing to VR. Therefore, in LAM-resistant CHB, ADV administration should be started before the flare-up of ALT elevation, especially in patients with severe liver disease such as cirrhosis.

In LAM-resistant patients, the HBV DNA level is low during the initial phase, but increases with time, leading to the onset of breakthrough hepatitis. Thus, in ADV therapy added to LAM treatment for LAM-resistant-CHB, the baseline HBV DNA level varies with the observation period after the emergence of LAM resistance. A previous report on Italian HBeAg-negative CHB patients showing LAM resistance revealed that patients with low viremia and normal ALT tended to respond to ADV therapy in addition to LAM treatment better than those with high viremia and abnormal ALT [17]. In the present study conducted in Japan, a genotype C-endemic area, such a close relationship between lower baseline HBV DNA and better therapeutic response was remarkable in

HBeAg-positive patients but not in HBeAg-negative ones. Our finding suggests that, in LAM-resistant CHB, ADV should be added before the HBV DNA begins to increase markedly, especially in HBeAg-positive patients.

In this study, none of the 75 patients showed virological breakthrough after the beginning of ADV administration. All displayed more than 1 log reduction of HBV DNA at 12 months of ADV treatment. This indicates that our patients may not have produced viruses resistant to both LAM and ADV. The emergence of resistant viruses has been reported to be rare in combination therapy using LAM and ADV for LAM-resistant CHB patients, although recent studies have found the existence of a virus resistant to both drugs [22, 23]. The rtA181V/T/S mutation has been reported to confer cross resistance to LAM and ADV [22, 23]. In ADV monotherapy for nucleos(t)ide analog-naïve CHB patients, the absence of HBV DNA reduction to <4 logcopies/ml at 24 weeks of treatment has been reported to be related to the higher emergence of a ADV-resistant virus [24], as is the case in LAM monotherapy [25]. In ADV therapy added to LAM treatment in LAM-resistant CHB patients, the poor response during the initial phase may lead to the development of virus resistance to LAM and ADV as well. From this point of view, the addition of ADV to ongoing LAM treatment before the elevation of HBV DNA may be beneficial in LAM-resistant CHB patients to avoid the development of a multi-drug-resistant virus. Recently, some investigators have reported that tenofovir disoproxil fumarate is effective against a virus resistant to both LAM and ADV [22, 23], but it has not yet been approved for clinical use.

Our results conclusively showed that, with ADV therapy added to LAM treatment for LAM-resistant CHB patients, lower baseline HBV DNA and negative HBeAg contributed to a better antiviral effect. After the emergence of LAM resistance, ADV should be added before the marked elevation of HBV DNA in order to attain better antiviral efficacy, especially in HBeAg-positive patients.

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