

patient's consent, or the patient was carefully followed until a diagnosis was possible with a definite observation by CT or angiography.

### Statistical analysis

Quantitative variables were expressed as mean  $\pm$  SD. The Kaplan–Meier method was used to calculate the cumulative incidence of HCC. The prognostic relevance of clinical variables and HCC incidence was evaluated by univariate analysis with log-rank test and by multivariate Cox's regression analysis. A value of  $P < 0.05$  (two-tailed) was considered to indicate significance. All calculations were performed with SPSS version 15.0J (SPSS, Chicago, IL, USA).

## RESULTS

### Baseline characteristics in patients treated with interferon therapy

THE BASELINE CLINICAL features of the enrolled patients are shown in Table 1. The mean age of the patients was  $55.8 \pm 10.9$  years, and 64% of the total cases were male. Two hundred and sixty-one patients (73%) were infected with HCV genotype 1 and had a viral load of more than  $10^5$  IU/ml. Liver biopsy was done for 320 cases and the ratio of patients with severe fibrosis (F3–4) diagnosed by the HAI score was more than 31%. The mean platelet count was  $14.8 \pm 5.1 \times 10^4/\mu\text{l}$ , and the ALT level was  $96.0 \pm 62.6$  IU/L. A sustained virological response (SVR) was achieved by 139 patients (34%) by combination therapy of IFN- $\alpha$ 2b

Table 1 Baseline characteristics in patients treated with interferon therapy

	All cases
Number of patients	403
Age	$55.8 \pm 10.9$
Gender (male/female)	257/145
Genotype and viral load (1H/non-1H)	261/97
Fibrosis (F0/1/2/3/4)	15/149/56/92/8
WBC ( $/\mu\text{l}$ )	$5113 \pm 1487$
Platelet ( $\times 10^4/\mu\text{l}$ )	$14.8 \pm 5.1$
ALT (IU/l)	$96.0 \pm 62.6$
IFN effect (SVR/TR/NR/cessation)	139/109/110/45

Data are number of patients, mean  $\pm$  standard deviation. Fibrosis stage is evaluated on a scale from 0 to 4 according to METAVIR's histological score. 1H, Genotype 1 and high viral load; non-1H, all except for 1H; ALT, alanine aminotransferase; IFN, interferon; NR, no response; SVR, sustained virological response; TR, transient response; WBC, white blood cells.

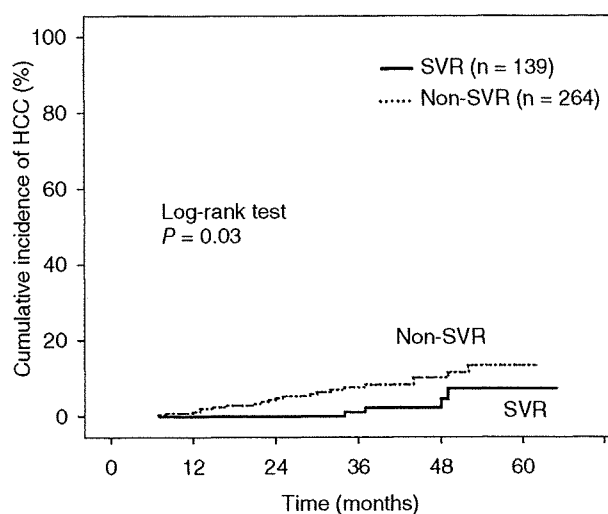


Figure 1 Cumulative incidence of development of hepatocellular carcinoma (HCC) according to treatment effect: (—) sustained virological response; (.....) non-sustained virological response.

plus ribavirin. According to an intent-to-treat analysis, 20% (51/261) of patients with HCV genotype 1 and a high viral load ( $\geq 100\text{KIU/mL}$ ) achieved SVR by the combination therapy, whereas 75% (73/97) of the patients with HCV genotype 2 or a low load showed SVR. The median observation period for all patients was  $36.5 \pm 14.8$  months with a range of 6 to 62 months from the end-point of IFN treatment.

### Cumulative incidence of development of HCC according to the treatment effect (SVR vs. non-SVR)

Figure 1 shows the Kaplan–Meier estimates of the cumulative HCC incidence according to the treatment effect (SVR vs. non-SVR). Twenty-five (6%) of the 403 enrolled patients developed HCC; four (2.9%) of the SVR group and 21 (8.0%) of the non-SVR group. The cumulative incidence rate of HCC was significantly lower in patients of the SVR group than in those of the non-SVR group ( $P = 0.03$ ).

### Cumulative incidence of HCC development according to the treatment effect (SVR vs. TR vs. NR vs. cessation)

Figure 2 shows the Kaplan–Meier estimates of the cumulative HCC incidence according to the treatment effect (SVR vs. TR vs. NR vs. cessation). Five patients (4.6%) of the TR group, nine (8.2%) of the NR group and seven (15.6%) of the cessation group developed

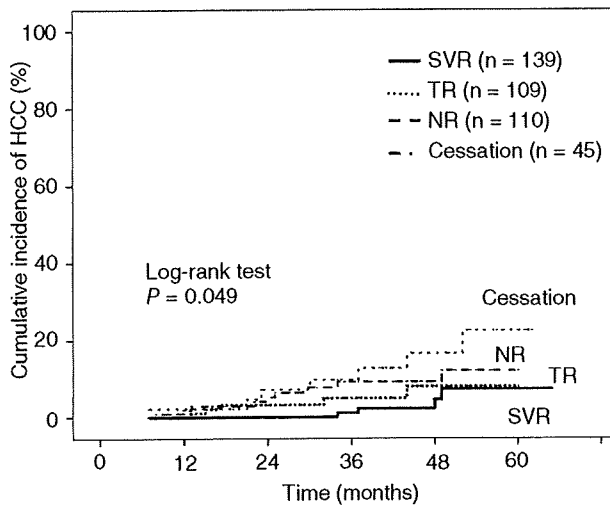


Figure 2 Cumulative incidence of hepatocellular carcinoma (HCC) development according to treatment effect: (—) sustained virological response; (.....) transient response group; (- -) no response; (- ·) cessation.

HCC. There was no significant difference in the cumulative incidence of HCC between the TR and NR groups ( $P = 0.394$ ). In contrast, the cumulative incidence rate of HCC was significantly lower in patients of the SVR group than in those of the NR group ( $P = 0.05$ ). These results indicate that treatment of the TR group with IFN- $\alpha$ -2b plus ribavirin therapy did not reduce HCC development when compared to the NR group.

### Risk factors for cumulative incidence of HCC development

Univariate analysis with the log-rank test showed that the following were significant risk factors for the development of HCC; older age ( $> 65$  years) ( $P = 0.01$ ), severe fibrosis ( $P = 0.006$ ), high platelet count ( $> 14 \times 10^4/\mu\text{l}$ ) ( $P = 0.017$ ) and non-SVR ( $P = 0.03$ ).

Stepwise multivariate analyses of these four variables were performed for all patients treated with combination therapy of IFN- $\alpha$ -2b plus ribavirin by Cox's regression analysis, as shown in Table 2. The analysis indicated the following factors as independent significant risk factors related to the development of HCC: older age (risk ratio, 3.23; 95% CI, 1.37-8.56;  $P = 0.006$ ), fibrosis staging (risk ratio, 1.69; 95% CI, 1.04-2.67;  $P = 0.033$ ) and non-SVR to IFN therapy (risk ratio, 3.57; 95% CI, 1.04-12.36;  $P = 0.044$ ).

### Cumulative incidence of HCC development according to average serum ALT levels after combination therapy

The average serum ALT levels in 134 patients (96.4%) of the SVR group were  $< 40$  IU/L after completion of the combination therapy, while 63 patients (24.4%) of the non-SVR group showed serum ALT levels of  $\geq 40$  IU/L. Figure 3 shows Kaplan-Meier estimates of the cumulative HCC incidence according to the average serum ALT levels after combination therapy. The cumulative incidence rate of HCC was significantly lower in patients with average serum ALT levels of  $< 40$  IU/L than with average serum ALT levels of  $\geq 40$  IU/L ( $P = 0.021$ ).

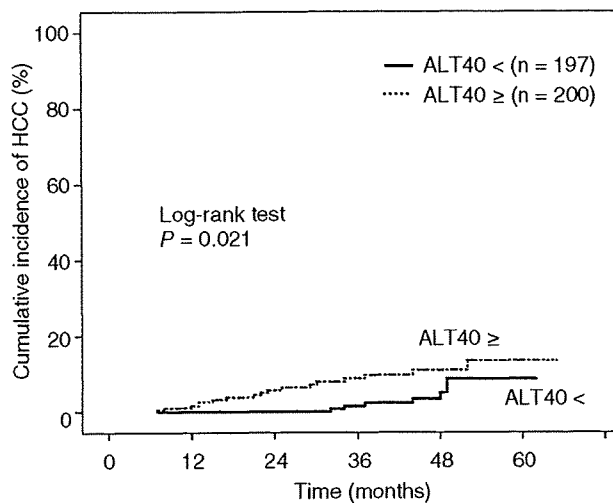
### Cumulative incidence of HCC development according to the treatment effect (SVR vs. non-SVR) in patients showing less than 40 IU/L average ALT levels after the combination therapy

Figure 4 shows Kaplan-Meier estimates of the cumulative HCC incidence according to the treatment effect (SVR vs. non-SVR) in patients who showed less than 40 IU/L average ALT levels after the combination therapy. There was no significant difference in the cumulative incidence rate of HCC between the SVR and non-SVR groups ( $P = 0.37$ ).

Table 2 Risk factors for cumulative incidence of HCC development

Variable	Category	Risk ratio	P value	95% CI
Gender	male	1		
	female	0.34	0.053	0.11-1.01
Age (years)	$65 <$	1		
	$65 \geq$	3.23	0.006	1.37-8.56
Fibrosis	F0/1/2/3/4	1.69	0.033	1.04-2.67
IFN therapy	Non-SVR	1		
	SVR	0.28	0.044	1.04-12.36

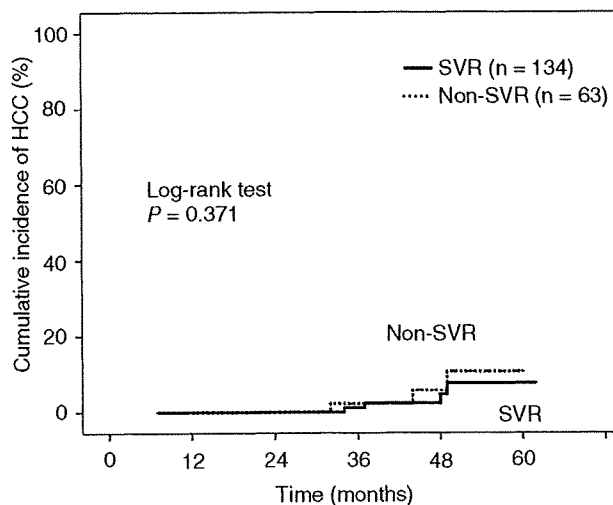
CI, confidence interval; IFN, interferon; SVR, sustained virological response.



**Figure 3** Cumulative incidence of HCC development according to average alanine aminotransferase (ALT) levels after the combination therapy (—) ALT < 40 IU/ml; (.....) ALT > 40 IU/ml.

## DISCUSSION

COMBINATION THERAPIES USING IFN- $\alpha$ -2b or Peg-IFN plus ribavirin have been proven to be more effective in treating for HCV infection than IFN monotherapy.<sup>15–17</sup> However, it has not been accurately



**Figure 4** Cumulative incidence of hepatocellular carcinoma (HCC) development according to the treatment effect in patients who showed less than 40 IU/L average alanine aminotransferase (ALT) levels after the combination therapy. (—) Sustained virological response; (.....) non-sustained virological response.

evaluated whether the combination therapies using IFN- $\alpha$ -2b or Peg-IFN plus ribavirin could reduce the development of HCC, and what the risk factors of HCC incidence were in patients infected with HCV. In this study, we retrospectively examined the incidence of HCC with IFN- $\alpha$ -2b plus ribavirin therapy to clarify the indicators of combination therapy for reducing HCC in patients infected with HCV. We also evaluated whether or not SVR or continuous normalization of ALT levels could reduce the risk of development of HCC.

Previous studies have demonstrated that IFN monotherapy has a preventive effect on the development of HCC, especially in patients with SVR.<sup>12–14</sup> In this study, using the combination of IFN- $\alpha$ -2b plus ribavirin, we obtained almost the same result for the SVR group treated with IFN- $\alpha$ -2b plus ribavirin therapy, which showed a significantly lower possibility of HCC development over a long-term period when compared with the non-SVR group. In contrast, we found no difference in the cumulative incidence of HCC between the TR and NR groups, while Kasahara *et al.* reported that the cumulative incidence of HCC in patients who achieved TR by IFN monotherapy was significantly lower than those with NR.<sup>13</sup> Recent reports have demonstrated that the combination therapy of IFN- $\alpha$ -2b plus ribavirin is able to induce a SVR in a significant proportion of patients with IFN monotherapy-resistant chronic hepatitis C,<sup>19,20</sup> suggesting that a viral relapse after IFN therapy is efficiently suppressed by combination with ribavirin. Since the combination therapy was a more effective treatment for HCV infection than IFN monotherapy<sup>15–17</sup> and there are fewer TR patients with combination therapy than with monotherapy, we speculate that not all, but quite a few patients of the TR group given IFN monotherapy corresponded to the SVR group given the combination therapy, and that the TR group given the combination therapy might have been included in the NR group of IFN monotherapy. This would mean that the “TR group given combination therapy” should be distinguished from the “TR group given IFN monotherapy”, and might explain why the results of this study were inconsistent with previous reports of the cumulative incidence of HCC in the TR group given IFN monotherapy being significantly lower than those with NR.<sup>13</sup>

The Kaplan–Meier method showed that older age (> 65 years), severe fibrosis (F2–4), high platelet count (>  $14 \times 10^4$ ) and non-SVR were significantly associated with the development of HCC. The Cox’s regression analysis indicated that older age, fibrosis staging and non-SVR to IFN therapy were significant risk factors related to the development of HCC. These results were

almost comparable with those of previous reports using IFN monotherapy<sup>12–14,21</sup> and IFN plus ribavirin combination therapy,<sup>22–24</sup> suggesting that the factors associated with the development of HCC are common among these treatments and that patients of older age, with advanced fibrosis and showing non-SVR to IFN therapy should be followed up carefully for longer periods, even if IFN therapy could be performed completely. In addition, four of the SVR group patients developed HCC at more than 6 months after the treatment, which means these patients need careful follow-up even if SVR has been achieved.<sup>25</sup>

The incidence of HCC has been reported to be lower in patients with normal ALT levels, even if serum HCV-RNA was positive 6 or 12 months after IFN monotherapy, when compared to those without a biochemical response,<sup>13,26,27</sup> suggesting that the aim of IFN therapy for patients infected with HCV should be not only HCV eradication, but also the achievement of a biochemical response in order to reduce the incidence of HCC. In this study, we divided the patients into two groups, one with persistently normal serum ALT levels and the other with elevated serum ALT levels based on “the average serum ALT levels” after completion of IFN therapy. We then evaluated the cumulative HCC incidence of each group using the Kaplan–Meier estimation. Our data showed that patients with continuous normalization of ALT levels have a lower possibility of HCC development than those showing elevated ALT after the combination therapy, suggesting that continuous normalization of ALT levels after the combination therapy is an important factor for reducing HCC development. Interestingly, based on the Kaplan–Meier estimates of the cumulative HCC incidence according to the treatment effect in patients who showed less than 40 IU/L average ALT levels after the combination therapy, we found no difference in HCC incidence rates between the SVR group and non-SVR group. Figure 1 shows that the combination therapy is strongly associated with a reduced incidence of HCC in the patients who attain SVR, which seems to be a means for achieving normalization of serum ALT levels in HCV patients. However, it was also shown that, even in the non-SVR group, patients with persistently normal serum ALT levels achieved a reduced risk of HCC development. Taken together, our aim of treatment for patients infected with HCV is to primarily completely eradicate HCV. Next, for the non-SVR group patients, we would speculate that maintaining normalization of ALT levels by some other treatments may prevent HCC development in HCV-infected patients with abnormal serum ALT levels even if

SVR is not achieved. Other treatments should be used to decrease serum ALT levels to below the upper limit of the normal range. Hopefully, the new treatments such as those with protease inhibitors can be helpful for these patients.<sup>28</sup>

Although IFN monotherapy in CHC patients has been demonstrated to be associated with reducing the incidence of HCC, especially in patients who attain SVR,<sup>12–14</sup> what actually occurs in IFN plus ribavirin combination therapy has not been clarified and the indicator for reducing HCC in patients infected with HCV has not been defined. We showed that this combination therapy could reduce the incidence of HCC and that older age, severe fibrosis and non-SVR were risk factors for HCC development. This therapy can increase the SVR patient ratio, and SVR or continuous normalization of ALT levels after combination therapy using IFN- $\alpha$ -2b plus ribavirin reduce the incidence of HCC in patients with HCV infection. Therefore, this therapy can not only avert the advance of the disease toward liver cirrhosis, but also decrease the risk of HCC. IFN plus ribavirin combination therapy is beneficial for HCV patients from both aspects. In conclusion, the present study shows that the attainment of SVR or continuous normalization of serum ALT levels induced by the combination therapy has a significantly beneficial effect on the clinical course of HCV patients by decreasing the incidence of HCC.

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## Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin

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**SUMMARY.** The impact of ribavirin exposure on virologic relapse remains controversial in combination therapy with pegylated interferon (Peg-IFN) and ribavirin for patients with chronic hepatitis C (CH-C) genotype 1. The present study was conducted to investigate this. Nine hundred and eighty-four patients with CH-C genotype 1 were enrolled. The drug exposure of each medication was calculated by averaging the dose actually taken. For the 472 patients who were HCV RNA negative at week 24 and week 48, multivariate logistic regression analysis showed that the degree of fibrosis ( $P = 0.002$ ), the timing of HCV RNA negativation ( $P < 0.001$ ) and the mean doses of ribavirin ( $P < 0.001$ ) were significantly associated with relapse, but those of Peg-IFN were not. Stepwise reduction of the ribavirin dose was associated with a stepwise increase in relapse rate from 11%

to 60%. For patients with complete early virologic response (c-EVR) defined as HCV RNA negativity at week 12, only 4% relapse was found in patients given  $\geq 12$  mg/kg/day of ribavirin and ribavirin exposure affected the relapse even after treatment week 12, while Peg-IFN could be reduced to 0.6  $\mu$ g/kg/week after week 12 without the increase of relapse rate. Ribavirin showed dose-dependent correlation with the relapse. Maintaining as high a ribavirin dose as possible ( $\geq 12$  mg/kg/day) during the full treatment period can lead to suppression of the relapse in HCV genotype 1 patients responding to Peg-IFN alpha-2b plus ribavirin, especially in c-EVR patients.

**Keywords:** chronic hepatitis C, drug exposure, pegylated interferon plus ribavirin, virologic relapse.

## INTRODUCTION

Combination therapy of pegylated interferon (Peg-IFN) plus ribavirin is very effective for patients with chronic hepatitis C

Abbreviations: CH-C, chronic hepatitis C; c-EVR, complete early virologic response; ETR, end-of-treatment virologic response; Hb, haemoglobin; HCV, hepatitis C virus; IFN, interferon; LVR, late virologic response; Peg-IFN, pegylated interferon; PP, per protocol; Plt, platelet; RVR, rapid virologic response; SVR, sustained virologic response; VR, virologic response; WBC, white blood cell.

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(CH-C). However, sustained virologic response (SVR) in current therapy occurs in only 40–50% of patients with hepatitis C virus (HCV) genotype 1 [1–4]. Also, SVR is reduced in patients with genotype 1 who require reduction of either Peg-IFN or ribavirin, although dose reduction has little influence on SVR in those with genotype 2 or 3 [1–3,5,6]. Therefore, it is important to clarify the degree to which these medications can be reduced without adversely affecting SVR in patients with CH-C genotype 1.

In an early report on the relationship between drug exposure and antiviral effect in patients with CH-C genotype 1, patients who received  $\geq 80\%$  of their total planned cumulative doses of Peg-IFN and ribavirin for  $\geq 80\%$  of the scheduled duration of therapy had an SVR of 51% compared with only 34% for patients who received lesser amounts of one or both

medications [7]. On the other hand, Shiffman *et al.* [8] recently reported that reducing ribavirin did not affect SVR as long as the dose of Peg-IFN was maintained, while reducing the Peg-IFN dose significantly reduced SVR. The results of these observations are consistent with respect to the effect of Peg-IFN on SVR. However, what is controversial is whether or not reducing the ribavirin dose affects the antiviral effect.

Adding ribavirin to either interferon (IFN) or Peg-IFN monotherapy for patients with CH-C genotype 1 has been shown to reduce the relapse rate in large randomized trials [1,2,9–11]. In detail, adding ribavirin to the usual IFN monotherapy (3MIU, three-times-weekly) in 48-week treatment raised the end-of-treatment virologic response (ETR) rate from approximately 30% to 50% and also lowered the relapse rate from mid-40% to approximately 20% [9–11]. Lindsay *et al.* [12] reported that Peg-IFN alpha-2b (Peg-IFN  $\alpha$ -2b) monotherapy (1.5  $\mu$ g/kg, once-weekly), as compared with IFN alpha-2b (IFN $\alpha$ -2b) monotherapy (3MIU, three-times-weekly), improved ETR (49% vs. 24%), but not the relapse rate (53% vs. 50%). In the trial of Peg-IFN alpha-2a (Peg-IFN  $\alpha$ -2a) plus ribavirin vs IFN  $\alpha$ -2b plus ribavirin or Peg-IFN  $\alpha$ -2a alone, the ETR rates were 69%, 52% and 59%, and the relapse rates were 19%, 15% and 52%, respectively [2]. These findings from large-scale trials indicate that the main role of ribavirin is to reduce relapse in the combination therapy with Peg-IFN, although ribavirin affects both ETR and relapse in combination therapy with the usual IFN.

In the present study, we tried to determine whether or not dose reduction of ribavirin (or Peg-IFN) has an effect on virologic relapse in Peg-IFN plus ribavirin treatment for patients with CH-C genotype 1.

## PATIENTS AND METHODS

### Patients

This study was a multicentre trial conducted by Osaka University Hospital and other institutions participating in the Osaka Liver Forum. A total of 984 patients with CH-C were enrolled in this study between December 2004 and September 2006, and treated with a combination of Peg-IFN  $\alpha$ -2b plus ribavirin. The baseline characteristics of the patients are shown in Table 1. All patients were Japanese infected with HCV genotype 1 and a viral load of more than  $10^5$  IU/mL. Patients were excluded from this study if they had decompensated cirrhosis or other forms of liver disease (alcohol liver disease, autoimmune hepatitis), coinfection with hepatitis B or anti-human immunodeficiency virus. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and informed consent was obtained from each patient.

### Treatment

All patients received Peg-IFN  $\alpha$ -2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL;

**Table 1** Baseline characteristics of patients and drug doses at start of treatment

Factor	Mean $\pm$ SD or <i>n</i>
<i>n</i>	984
Age (years)	56.3 $\pm$ 10.1
Sex (male/female)	555/429
Body weight (kg)	61.8 $\pm$ 11.5
History of IFN treatment	575/409 (160/182)
Naïve/experienced	
(relapser/nonresponder)*	
White blood cells (/mm <sup>3</sup> )	5052 $\pm$ 1550
Neutrophils (/mm <sup>3</sup> )	2577 $\pm$ 1092
Red blood cells ( $\times 10^4$ /mm <sup>3</sup> )	442 $\pm$ 47
Haemoglobin (g/dL)	14.1 $\pm$ 1.4
Platelets ( $\times 10^4$ /mm <sup>3</sup> )	15.9 $\pm$ 5.5
AST (IU/L)	66 $\pm$ 45
ALT (IU/L)	79 $\pm$ 61
Serum HCV RNA (kIU/mL) <sup>†</sup>	1600
Histology (METAVIR) <sup>‡</sup>	
Fibrosis: 0/1/2/3/4	49/314/197/105/18
Activity: 0/1/2/3	23/329/304/27
Peg-IFN dose ( $\mu$ g/kg/week)	1.45 $\pm$ 0.17
Ribavirin dose (mg/kg/day)	11.4 $\pm$ 1.6

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. <sup>†</sup>Data shown are median values. <sup>‡</sup>301 missing.

Schering-Plough) for the duration of the study of 48 weeks. As a starting dose, Peg-IFN  $\alpha$ -2b was given subcutaneously once weekly at a dosage of 60–150  $\mu$ g/kg based on body weight (body weight 35–45 kg, 60  $\mu$ g; 46–60 kg, 80  $\mu$ g; 61–75 kg, 100  $\mu$ g; 76–90 kg, 120  $\mu$ g; 91–120 kg, 150  $\mu$ 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 the manufacturer's drug information available in Japan.

### Dose reduction and discontinuance

Dose modification also followed, as a rule, the manufacturer's drug information according to the intensity of the haematologic adverse effects. The dose of Peg-IFN  $\alpha$ -2b was reduced to 50% of the assigned dose when the white blood cell (WBC) count was below 1500/mm<sup>3</sup>, the neutrophil count below 750/mm<sup>3</sup> or the platelet (Plt) count below  $8 \times 10^4$ /mm<sup>3</sup>, and was discontinued when the WBC count was below 1000/mm<sup>3</sup>, the neutrophil count below 500/mm<sup>3</sup> or the Plt count below  $5 \times 10^4$ /mm<sup>3</sup>. Ribavirin was also reduced from 1000 mg to 600 mg, 800 mg to 600 mg, or 600 mg to 400 mg when the haemoglobin (Hb)

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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -2b and ribavirin were calculated individually as averages on the basis of body weight at baseline: Peg-IFN  $\alpha$ -2b expressed as  $\mu\text{g}/\text{kg}/\text{week}$ , ribavirin expressed as  $\text{mg}/\text{kg}/\text{day}$ .

#### *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  $\alpha$ -2b exposure into five categories (up to 0.6  $\mu\text{g}/\text{kg}$ ; from 0.6 to less than 0.9  $\mu\text{g}/\text{kg}$ ; from 0.9 to less than 1.2  $\mu\text{g}/\text{kg}$ ; from 1.2 to less than 1.5  $\mu\text{g}/\text{kg}$ ; from 1.5  $\mu\text{g}/\text{kg}$ ) and ribavirin exposure into five categories (up to 6  $\text{mg}/\text{kg}$ ; from 6 to less than 8  $\text{mg}/\text{kg}$ ; from 8 to less than 10  $\text{mg}/\text{kg}$ ; from 10 to less than 12  $\text{mg}/\text{kg}$ ; from 12  $\text{mg}/\text{kg}$ ).

#### *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 $\alpha$ -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  $\alpha$ -2b actually taken during the full treatment period by each patient was 1.32  $\mu\text{g}/\text{kg}/\text{week}$  (range, 0.49–2.16  $\mu\text{g}/\text{kg}/\text{week}$ ; median, 1.38  $\mu\text{g}/\text{kg}/\text{week}$ ) and that of ribavirin was 9.8  $\text{mg}/\text{kg}/\text{day}$  (range, 3.3–16.2  $\text{mg}/\text{kg}/\text{day}$ ; median, 10.1  $\text{mg}/\text{kg}/\text{day}$ ) 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  $\alpha$ -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



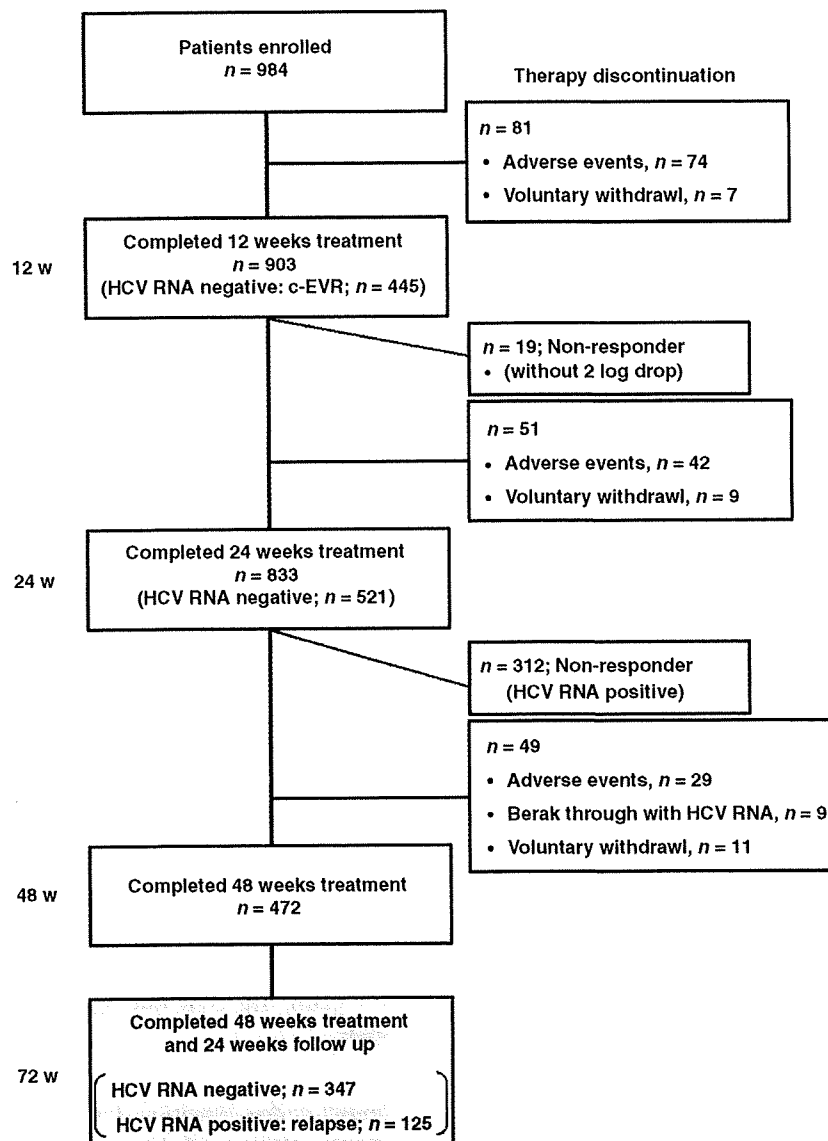


Fig. 1 Flow of patients throughout the study.

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  $\geq 12$  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  $\alpha$ -2b: the group given  $\geq 1.4$   $\mu\text{g}/\text{kg}/\text{week}$  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  $\geq 1.4$   $\mu\text{g}/\text{kg}/\text{week}$  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  $\alpha$ -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

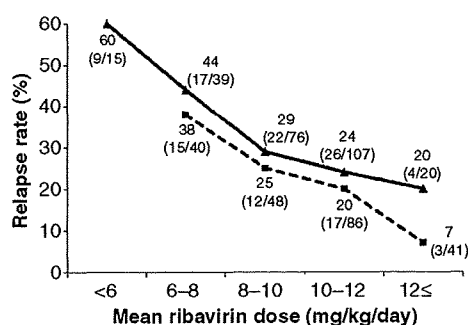
**Table 2** Factors associated with relapse among the patients with virologic response

(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 <sup>3</sup> )	5335 ± 1517	5075 ± 1428	0.08	
Neutrophils (/mm <sup>3</sup> )	2797 ± 1143	2625 ± 1021	0.17	
Red blood cells (×10 <sup>4</sup> /mm <sup>3</sup> )	450 ± 45	446 ± 50	0.25	
Haemoglobin (g/dL)	14.3 ± 1.4	14.2 ± 1.5	0.45	
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )	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) <sup>†</sup>				
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) <sup>‡</sup>	1.33 ± 0.26	1.27 ± 0.29	0.07	
Ribavirin dose (mg/kg/day) <sup>‡</sup>	10.1 ± 1.9	9.1 ± 2.1	<0.001	
Virologic response <sup>§</sup> : c-EVR/LVR	316/31	75/50	<0.001	
(b) Multivariate analysis				
Factor	Category	Odds ratio	95% CI	P value
Platelets	By 1 × 10 <sup>4</sup> /mm <sup>3</sup>	–	–	NS
Fibrosis <sup>¶</sup>	0–2/3–4	1/3.192	1.515–6.725	0.002
Ribavirin dose <sup>‡</sup>	By 1 mg/kg/day	0.790	0.696–0.896	<0.001
Virologic response <sup>§</sup>	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. <sup>†</sup>137 missing. <sup>‡</sup>Mean doses during 0–48 weeks. <sup>§</sup>The timing of HCV RNA negativation.

<sup>¶</sup>METAVIR fibrosis score.



**Fig. 2** Relapse rate according to Peg-IFN  $\alpha$ -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  $\mu$ g/kg/week; (--- $\blacksquare$ ) Group with the mean Peg-IFN dose  $\geq$ 1.4  $\mu$ g/kg/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  $\alpha$ -2b-dose groups ( $P = 0.17$ ).

of ribavirin. In contrast, the relapse rate was not affected by the dose of Peg-IFN  $\alpha$ -2b when the patients were given more than 0.9  $\mu$ g/kg/week of Peg-IFN  $\alpha$ -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  $\alpha$ -2b or ribavirin (prevalence of patients: the mean dose of Peg-IFN  $\alpha$ -2b: <0.9: 0.9–1.2: 1.2–1.5: 1.5  $\mu$ g/kg/week  $\leq$ : 7: 17: 34: 42%, the mean dose of ribavirin; <8: 8–10: 10–12: 12 mg/kg/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 mg/kg/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 ( $\mu\text{g}/\text{kg}/\text{week}$ ) <sup>†</sup>	Ribavirin dose (mg/kg/day)*								Total	
	12 $\leq$	10–12		8–10		<8				
$\geq 1.5$	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 ( $\mu\text{g}/\text{kg}/\text{week}$ ) <sup>§</sup>	Ribavirin dose (mg/kg/day) <sup>‡</sup>								Total	
	12 $\leq$	10–12		8–10		<8				
$\geq 1.5$	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. <sup>†</sup> $P = 0.08$  for comparison of the four Peg-IFN groups. <sup>‡</sup> $P = 0.03$  for comparison of the four ribavirin groups. <sup>§</sup> $P = 0.57$  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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\geq 10$  mg/kg/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,  $10.5 \pm 2.1$  mg/kg/day vs relapser,  $8.8 \pm 2.3$  mg/kg/day;  $P = 0.007$ ). Among patients with advanced fibrosis (score 3–4),

the relapse rate in patients given  $\geq 10$  mg/kg/day of the average ribavirin dose was significantly low (36%, 9/25) in comparison with that in patients given  $< 10$  mg/kg/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  $\alpha$ -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

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)

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  $\alpha$ -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  $\alpha$ -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  $\geq 10$  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  $\alpha$ -2b dose when the patients were given more than  $0.9 \mu\text{g}/\text{kg}/\text{week}$  of Peg-IFN  $\alpha$ -2b. Among the patients with c-EVR, none with RVR had a relapse and all attained SVR irrespective of the dose of Peg-IFN  $\alpha$ -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  $\geq 10$  mg/kg/day of the ribavirin dose during the first 12 weeks, while the Peg-IFN  $\alpha$ -2b dose after week 12 could be reduced without any increase in relapse rate in patients given more than  $0.6 \mu\text{g}/\text{kg}/\text{week}$  of the average dose of Peg-IFN  $\alpha$ -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  $\geq 12$  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 ( $\geq 12$  mg/kg/day) of ribavirin during the full treatment period could strongly suppress the relapse in such patients, while Peg-IFN  $\alpha$ -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 $\alpha$ -2b) affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with Peg-IFN $\alpha$ -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  $\alpha$ -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  $\geq 1.2$   $\mu\text{g}/\text{kg}/\text{week}$  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  $\alpha$ -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  $56.3 \pm 10.1$  years, and 56% were males. The mean serum alanine aminotransferase level was  $79 \pm 61$  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)	$56.3 \pm 10.1$
Sex: male/female	555/429
Body weight (kg)	$61.8 \pm 11.5$
History of interferon treatment	
Naïve/experienced	575/409(160/182)
(relapser/nonresponder)*	
White blood cells (per mm <sup>3</sup> )	$5052 \pm 1550$
Neutrophils (per mm <sup>3</sup> )	$2577 \pm 1092$
Red blood cells ( $\times 10^4$ /mm <sup>3</sup> )	$442 \pm 47$
Haemoglobin (g/dL)	$14.1 \pm 1.4$
Platelets ( $\times 10^4$ /mm <sup>3</sup> )	$15.9 \pm 5.5$
AST (IU/L)	$66 \pm 45$
ALT (IU/L)	$79 \pm 61$
Serum HCV RNA (kIU/mL) <sup>†</sup>	1600
Histology (METAVIR) <sup>‡</sup>	
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. <sup>†</sup>Data shown are median values. <sup>‡</sup>301 missing.

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

### Treatment

All patients received Peg-IFN  $\alpha$ -2b (PEGINTRON; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (REBETOL; Schering-Plough) for the duration of the study of 48 weeks. Peg-IFN  $\alpha$ -2b was given subcutaneously once weekly at a dosage of 60–150  $\mu$ g/kg based on body weight (body weight 35–45 kg, 60  $\mu$ g; 46–60 kg, 80  $\mu$ g; 61–75 kg, 100  $\mu$ g; 76–90 kg, 120  $\mu$ g; 91–120 kg, 150  $\mu$ g) and ribavirin was given orally twice a day at a total dose of 600–1000 mg/day based on body weight (body weight  $\leq 60$  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  $\alpha$ -2b was reduced to 50% of the assigned dose if the white blood cell (WBC) count declined to  $< 1500$ /mm<sup>3</sup>, the neutrophil count to  $< 750$ /mm<sup>3</sup> or the platelet (Plt) count to  $< 8 \times 10^4$ /mm<sup>3</sup>, and was discontinued if the WBC count declined to  $< 1000$ /



mm<sup>3</sup>, the neutrophil count to <500/mm<sup>3</sup> or the Plt count to <5 × 10<sup>4</sup>/mm<sup>3</sup>. 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  $\alpha$ -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 IU/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  $\alpha$ -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  $\alpha$ -2b expressed as  $\mu$ 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  $\alpha$ -2b dose (average dose per body weight and per week) was classified into five categories (up to 0.6  $\mu$ g/kg; from 0.6 to <0.9  $\mu$ g/kg; from 0.9 to <1.2  $\mu$ g/kg; from 1.2 to <1.5  $\mu$ g/kg; from 1.5  $\mu$ 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 $\alpha$ -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  $\alpha$ -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 $\alpha$ -2b and ribavirin on c-EVR*

The mean dose of Peg-IFN  $\alpha$ -2b actually taken during the first 12 weeks by each patient was 1.33  $\mu$ g/kg/week (range 0.41–2.16  $\mu$ g/kg/week; median 1.40  $\mu$ 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  $\alpha$ -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 <sup>3</sup> )	5336 ± 1536	4818 ± 1547	<0.001
Neutrophils (per mm <sup>3</sup> )	2789 ± 1133	2398 ± 1038	<0.001
Red blood cells (×10 <sup>4</sup> /mm <sup>3</sup> )	450 ± 46	435 ± 49	<0.001
Haemoglobin (g/dL)	14.3 ± 1.4	13.9 ± 1.4	<0.001
Platelets (×10 <sup>4</sup> /mm <sup>3</sup> )	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) <sup>†</sup>			
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) <sup>‡</sup>	1.39 ± 0.22	1.28 ± 0.30	<0.001
Ribavirin dose (mg/kg/day) <sup>‡</sup>	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. <sup>†</sup>272 missing. <sup>‡</sup>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 <sup>3</sup>	1.017	1.002–1.033	0.03
Red blood cells	by 1 × 10 <sup>4</sup> /mm <sup>3</sup>	–	–	NS
Haemoglobin	by 1 g/dL	–	–	NS
Platelets	by 1 × 10 <sup>4</sup> /mm <sup>3</sup>	1.051	1.014–1.088	<0.01
AST	by 1 IU/L	–	–	NS
Fibrosis*	0–2/3–4	–	–	NS
Peg-IFN dose <sup>†</sup>	by 0.1 µg/kg/week	1.079	1.011–1.151	0.02
Ribavirin dose <sup>†</sup>	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. <sup>†</sup>Mean doses during 0–12 weeks.

ate analysis were evaluated by multivariate logistic regression analysis. The mean dose of Peg-IFN  $\alpha$ -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  $\geq 1.5$  and 1.2–1.5 µg/kg/week of Peg-IFN  $\alpha$ -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  $\alpha$ -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  $\geq 1.2$  µg/kg/week of Peg-IFN  $\alpha$ -2b was significantly higher than that of the patients with  $< 1.2$  µg/kg/week [ $\geq 1.2$  µg/kg/week, 55% (383/696) vs  $< 1.2$  µg/kg/week, 30% (62/207),  $P < 0.0001$ ].

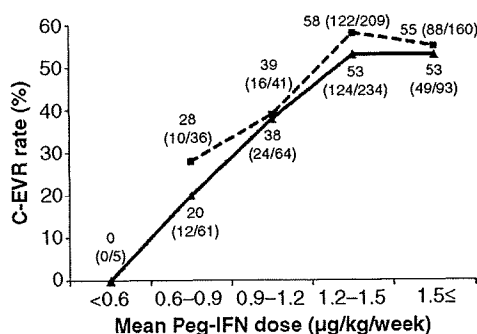
Next, we analysed the impact of Peg-IFN  $\alpha$ -2b on c-EVR in stratified analysis according to ribavirin dose. Figure 1 shows the relationship of c-EVR and the degree of Peg-IFN  $\alpha$ -2b exposure for two groups of ribavirin doses: the group with  $\geq 10.6$  mg/kg/day of ribavirin and that with  $< 10.6$  mg/kg/day (10.6 mg/kg/day was the median value). In either group, the mean dose of Peg-IFN  $\alpha$ -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  $\alpha$ -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 $\alpha$ -2b dose ( $\mu$ g/kg/week),*				Total
	$\geq 1.5$	1.2–1.5	0.9–1.2	$< 0.9$	
$\geq 12$	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  $\alpha$ -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  $\geq 10.6$  mg/kg/day. The Peg-IFN  $\alpha$ -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 $\alpha$ -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  $\alpha$ -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 $\alpha$ -2b dose ( $\mu$ g/kg/week)	$\geq 80\%$	60–80%	$< 60\%$	Total
$\geq 1.2$	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  $\geq 1.2$   $\mu$ g/kg/week vs  $< 1.2$   $\mu$ g/kg/week among the patients with more than 80% of the total prescribed dose of Peg-IFN  $\alpha$ -2b. \*\* $P = 0.01$ ; patients with  $\geq 1.2$   $\mu$ g/kg/week vs  $< 1.2$   $\mu$ g/kg/week among the patients with more than 60–80% of the total prescribed dose of Peg-IFN  $\alpha$ -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  $\alpha$ -2b. Among patients given  $\geq 80\%$  of the prescribed dose of Peg-IFN  $\alpha$ -2b, the c-EVR rate was significantly lower in patients given  $< 1.2$   $\mu$ g/kg/week of Peg-IFN  $\alpha$ -2b than those given  $\geq 1.2$   $\mu$ 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  $\alpha$ -2b, if they were given  $\geq 1.2$   $\mu$ g/kg/week of Peg-IFN  $\alpha$ -2b, a higher c-EVR rate was attained in comparison with those given  $< 1.2$   $\mu$ g/kg/week (71% vs 38%,  $P = 0.01$ ); the c-EVR rate in patients given 60–80% of the prescribed dose and  $\geq 1.2$   $\mu$ g/kg/week of Peg-IFN  $\alpha$ -2b was not inferior to that in patients given  $\geq 80\%$  of the prescribed dose and  $\geq 1.2$   $\mu$ g/kg/week of Peg-IFN  $\alpha$ -2b.

#### Impact of dose exposure of Peg-IFN $\alpha$ -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  $\alpha$ -2b and ribavirin treatment were regarded as non-responders and stopped treatment [11]. We analysed the

relationship between the dose exposure to Peg-IFN  $\alpha$ -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  $\alpha$ -2b during the 12 weeks of treatment: patients given  $\geq 1.5$ , 1.2–1.5, 0.9–1.2 and  $< 0.9$   $\mu\text{g}/\text{kg}/\text{week}$  of Peg-IFN  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\geq 1.2$   $\mu\text{g}/\text{kg}$  of Peg-IFN  $\alpha$ -2b than in those given  $< 1.2$   $\mu\text{g}/\text{kg}$  of Peg-IFN  $\alpha$ -2b for aged patients over 60 years of age ( $\geq 1.2$   $\mu\text{g}/\text{kg}$ ; 46% vs  $< 1.2$   $\mu\text{g}/\text{kg}$ ; 28%,  $P < 0.01$ ) or for patients with a low Plt value ( $< 12 \times 10^4/\text{mm}^3$ ) ( $\geq 1.2$   $\mu\text{g}/\text{kg}$ ; 45% vs  $< 1.2$   $\mu\text{g}/\text{kg}$ ; 22%,  $P < 0.001$ ). Therefore, a marked dose reduction of Peg-IFN  $\alpha$ -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  $\geq 1.2$   $\mu\text{g}/\text{kg}/\text{week}$  of Peg-IFN  $\alpha$ -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  $\alpha$ -2b decreased. Therefore, the dose of Peg-IFN  $\alpha$ -2b should be maintained as high as possible even in patients who have to reduce Peg-IFN  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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  $\alpha$ -2b was low (32%) even in those who received  $\geq 80\%$  of the total planned doses of Peg-IFN  $\alpha$ -2b. If given  $\geq 1.2$   $\mu\text{g}/\text{kg}/\text{week}$  of Peg-IFN  $\alpha$ -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  $\alpha$ -2b (54%). This means that patients whose starting dose of Peg-IFN  $\alpha$ -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  $\geq 1.5$   $\mu\text{g}/\text{kg}/\text{week}$  of Peg-IFN  $\alpha$ -2b at the start can have their dosage reduced to 80% ( $\geq 1.2$   $\mu\text{g}/\text{kg}/\text{week}$ ) 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  $\alpha$ -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  $\alpha$ -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