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 (μg/kg/week)†	Ribavirin dose (mg/kg/day)*									
	12≤		10–12		8–10		<8		Total	
≥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 (μg/kg/week)§	Ribavirin dose (mg/kg/day)‡									
	12≤		10–12		8–10		<8		Total	
≥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 ≥1.2 μg/kg/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 μg/kg/week during 12–48 weeks (17%, 7/41) and those without reduction of Peg-IFN α-2b (average dose ≥1.2 μg/kg/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 μg/kg/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 ≥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 ± 2.1 mg/kg/day *vs* relapser, 8.8 ± 2.3 mg/kg/day; P = 0.007). Among patients with advanced fibrosis (score 3–4), the relapse rate in patients given ≥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 ± 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 negativiation [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 negativiation cannot differentiate it from the strong influence of the Peg-IFN effect, which affects HCV RNA negativiation 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 negativiation. We found exposure to ribavirin dose, timing of HCV RNA negativiation 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 μg/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 μg/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-Americans 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.

ACKNOWLEDGEMENTS AND DISCLOSURES

Other institutions and participants in the Osaka Liver Forum are: Osaka General Medical Center, A Inoue; Toyonaka Municipal Hospital, M Inada; Sumitomo Hospital, A Yamada; Kinki Central Hospital of Mutual Aid Association of Public School Teachers, E Hayashi; Yao Municipal Hospital, H Fukui; Otemae Hospital, Y Doi; Itami City Hospital, T Kashihara; Ashiya Municipal Hospital, K Kiriyama; National Hospital Organization Minami Wakayama Medical Center, K Fujimoto; Saiseikai Senri Hospital, K Suzuki; Nishinomiya Municipal Central Hospital, H Ogawa; Kano General Hospital, S Kubota; Saso Hospital, M Nishiuchi; and Osaka Kaisei Hospital, N Imaizumi.

This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from Ministry of Health Labour and Welfare of Japan, and Scientific Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–965.

2 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.

3 Hadziyannis SJ, Sette Jr H, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; 140: 346–355.
4 Hayashi N, Takehara T. Antiviral therapy for chronic hepatitis C: past, present, and future. *J Gastroenterol* 2006; 41: 17–27.
5 Zeuzem S, Hultcrantz R, Bourliere M *et al.* Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004; 40: 993–999.
6 Ferenci P, Brunner H, Laferl H *et al.* A randomized, prospective trial of ribavirin 400 mg day-1 versus 800 mg day-1 in combination with peginterferon alfa-2a in hepatitis C virus genotypes 2 and 3. *Hepatology* 2008; 47: 1816–1823.
7 McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; 123: 1061–1069.
8 Shiffman ML, Ghany MG, Morgan TR *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; 132: 103–112.
9 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1485–1492.
10 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; 352: 1426–1432.
11 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1493–1499.
12 Lindsay KL, Trepo C, Heintges T *et al.* A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; 34: 395–403.
13 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; 38: 645–652.
14 Shiffman ML, Di Bisceglie AM, Lindsay KL *et al.* Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004; 126: 1015–1023.
15 Reddy KR, Shiffman ML, Morgan TR *et al.* Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007; 5: 124–129.
16 Lodato F, Azzaroli F, Brillanti S *et al.* Higher doses of peginterferon alpha-2b administered twice weekly improve sustained virological response in difficult-to-treat patients with chronic hepatitis C: results of a pilot randomized study. *J Viral Hepat* 2005; 12: 536–542.
17 Lindahl K, Stahle L, Bruchfeld A, Schvarcz R. High-dose ribavirin in combination with standard dose peginterferon for treatment of patients with chronic hepatitis C. *Hepatology* 2005; 41: 275–279.
18 Bronowicki JP, Ouzan D, Asselah T *et al.* Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology* 2006; 131: 1040–1048.
19 Oze T, Hiramatsu N, Yakushijin T *et al.* Peginterferon alfa-2b affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with pegylated interferon alfa-2b plus ribavirin. *J Viral Hepat*, in press.
20 Berg T, von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. *Gastroenterology* 2006; 130: 1086–1097.
21 Sanchez-Tapias JM, Diago M, Escartin P *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; 131: 451–460.
22 Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; 46(6): 1688–1694.
23 Mangia A, Minerva N, Bacca D *et al.* Individualized treatment duration for hepatitis C genotype 1 patients: a randomized controlled trial. *Hepatology* 2008; 47: 43–50.
24 Shiffman ML, Salvatore J, Hubbard S *et al.* Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology* 2007; 46: 371–379.
25 Layden-Almer JE, Ribeiro RM, Wiley T, Perelson AS, Layden TJ. Viral dynamics and response differences in HCV-infected African American and white patients treated with IFN and ribavirin. *Hepatology* 2003; 37: 1343–1350.
26 Jacobson IM, Brown RS Jr, McCone J *et al.* Impact of weight-based ribavirin with peginterferon alfa-2b in African Americans with hepatitis C virus genotype 1. *Hepatology* 2007; 46: 982–990.
27 Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. *Lancet* 1991; 337: 1058–1061.
28 Di Bisceglie AM, Shindo M, Fong TL *et al.* A pilot study of ribavirin therapy for chronic hepatitis C. *Hepatology* 1992; 16: 649–654.
29 Hiramatsu N, Hayashi N, Haruna Y *et al.* Immunohistochemical detection of hepatitis C virus-infected hepatocytes in chronic liver disease with monoclonal antibodies to core, envelope and NS3 regions of the hepatitis C virus genome. *Hepatology* 1992; 16: 306–311.
30 Miyatake H, Kanto T, Inoue M *et al.* Impaired ability of interferon-alpha-primed dendritic cells to stimulate Th1-type CD4 T-cell response in chronic hepatitis C virus infection. *J Viral Hepat* 2007; 14: 404–412.
31 Itose I, Kanto T, Inoue M *et al.* Involvement of dendritic cell frequency and function in virological relapse in pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C patients. *J Med Virol* 2007; 79: 511–521.

Journal of Viral Hepatitis, 2009, 16, 578–585 doi:10.1111/j.1365-2893.2009.01116.x

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

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

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

Received November 2008; accepted for publication December 2008

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 μg/kg/week of Peg-IFN, and declined to 38% at 0.9–1.2 μg/kg/week, and 22% in patients given <0.9 μg/kg/week ($P < 0.0001$). Even with stratified analysis according to ribavirin dose, the dose-dependent effect of Peg-IFN on c-EVR was observed, and similar c-EVR rates were obtained if the dose categories of Peg-IFN were the same. Furthermore, the mean dose of Peg-IFN during the first 12 weeks affected HCV RNA negativity at week 24 ($P < 0.0001$) and SVR ($P < 0.0001$) in a dose-dependent manner. Our results suggest that Peg-IFN was dose-dependently correlated with c-EVR, independently of ribavirin dose. Thus, maintaining the Peg-IFN dose as high as possible during the first 12 weeks can yield HCV RNA negativity and higher c-EVR rates, leading to better SVR rates in patients with CH-C genotype 1.

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

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

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

*These authors contributed equally to this work.

INTRODUCTION

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

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

The relationship between drug exposure and anti-viral effect has been reported in several papers [2,11–15]. McHutchison *et al.* [12] demonstrated that the SVR rate in patients who received ≥80% of their total planned doses of Peg-IFN and ribavirin for ≥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 Declaration of Helsinki and informed consent was obtained from each patient.

Table 1 Baseline characteristics of patients

Factor	Mean ± SD or number
n	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 (relapser/nonresponder)*	575/409(160/182)
White blood cells (per mm^3)	5052 ± 1550
Neutrophils (per mm^3)	2577 ± 1092
Red blood cells ($\times 10^4/mm^3$)	442 ± 47
Haemoglobin (g/dL)	14.1 ± 1.4
Platelets ($\times 10^4/mm^3$)	15.9 ± 5.5
AST (IU/L)	66 ± 45
ALT (IU/L)	79 ± 61
Serum HCV RNA (kIU/mL)†	1600
Histology (METAVIR)‡	
Fibrosis; 0/1/2/3/4	49/314/197/105/18
Activity; 0/1/2/3	23/329/304/27

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.
*Viral response to previous treatment was unknown in 57 patients, and 10 patients had discontinued treatment. †Data shown are median values. ‡301 missing.

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 μg/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/mm^3, the neutrophil count to <750/mm^3 or the platelet (Plt) count to <8 × $10^4/mm^3$, and was discontinued if the WBC count declined to <1000/

mm^3, the neutrophil count to $<500/mm^3$ or the Plt count to $<5 \times 10^4/mm^3$. 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 haematopoetic 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 Diagnotics, 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 μg/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 μg/kg; from 0.6 to <0.9 μg/kg; from 0.9 to <1.2 μg/kg; from 1.2 to <1.5 μg/kg; from 1.5 μg/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 ± 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 μg/kg/week (range 0.41–2.16 μg/kg/week; median 1.40 μg/kg/week) and that of ribavirin was 10.4 mg/kg/day (range 2.9–16.2 mg/kg/day; median 10.6 mg/kg/day).

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

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

Factor	c-EVR (+)	c-EVR (−)	*P*-value
n	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 ($\times 10^4/mm^3$)	450 ± 46	435 ± 49	<0.001
Haemoglobin (g/dL)	14.3 ± 1.4	13.9 ± 1.4	<0.001
Platelets ($\times 10^4/mm^3$)	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 $1 \times 10^4/mm^3$	–	–	NS
Haemoglobin	by 1 g/dL	–	–	NS
Platelets	by $1 \times 10^4/mm^3$	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 (μg/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.

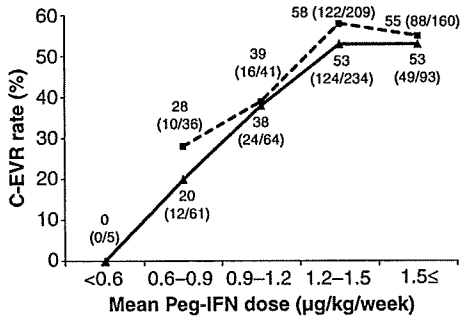

Fig. 1 Complete-EVR rate according to pegylated interferon alpha-2b (Peg-IFN α-2b) and ribavirin doses during weeks 0–12 for patients who completed 12 weeks of treatment. (-▲-) Group with the mean ribavirin dose <10.6 mg/kg/day. (··■··) Group with the mean ribavirin dose ≥10.6 mg/kg/day. The Peg-IFN 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 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 ≥80% of the prescribed dose of Peg-IFN α-2b, the c-EVR rate was significantly lower in patients given <1.2 μg/kg/week of Peg-IFN α-2b than those given ≥1.2 μg/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 μg/kg/week of Peg-IFN α-2b, a higher c-EVR rate was attained in comparison with those given <1.2 μg/kg/week (71% *vs* 38%, $P = 0.01$); the c-EVR rate in patients given 60–80% of the prescribed dose and ≥1.2 μg/kg/week of Peg-IFN α-2b was not inferior to that in patients given ≥80% of the prescribed dose and ≥1.2 μg/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

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 (μg/kg/week)	≥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 μg/kg/week *vs* <1.2 μg/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 μg/kg/week *vs* <1.2 μg/kg/week among the patients with more than 60–80% of the total prescribed dose of Peg-IFN α-2b.

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 μg/kg/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 μg/kg of Peg-IFN α-2b than in those given <1.2 μg/kg of Peg-IFN α-2b for aged patients over 60 years of age (≥1.2 μg/kg; 46% *vs* <1.2 μg/kg; 28%, $P < 0.01$) or for patients with a low Plt value ($<12 \times 10^4/mm^3$)(≥1.2 μg/kg; 45% *vs* <1.2 μg/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 μg/kg/week of Peg-IFN α-2b is desirable as a starting dose for achieving c-EVR even in these patients: that of <1.2 μg/kg/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 μg/kg/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 μg/kg, but the usual dose for the individual patient is from 1.28 to 1.76 μg/kg/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 μg/kg/week of Peg-IFN α-2b was low (32%) even in those who received ≥80% of the total planned doses of Peg-IFN α-2b. If given ≥1.2 μg/kg/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 ≥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 μg/kg/week should not have their dosage reduced to 80% of the planned dose (<1.2 μg/kg/week) in order to have a higher probability of c-EVR, while those given ≥1.5 μg/kg/week of Peg-IFN α-2b at the start can have their dosage reduced to 80% (≥1.2 μg/kg/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 μg/kg/week) during the first 12 weeks can yield higher c-EVR rates, leading to better treatment outcomes for patients with CH-C genotype 1.

ACKNOWLEDGMENTS AND DISCLOSURES

Other institutions and participants in the Osaka Liver Forum are: K Katayama, Osaka Koseinenkin Hospital; H Fukui, Yao Municipal Hospital; Y Doi, Otemae Hospital; A Kaneko, NTT West Osaka Hospital; T Kashihara, Itami City Hospital; K Kiriyama, Ashiya Municipal Hospital; T Nagase, Suita Municipal Hospital; M Inada, Toyonaka Municipal Hospital; K Fujimoto, National Hospital Organization Minami Wakayama Medical Center; K Suzuki, Saiseikai Senri Hospital; H Ogawa, Nishinomiya Municipal Central Hospital; S Kubota, Kano General Hospital; M Nishiuchi, Saso Hospital; and N Imaizumi, Osaka Kaisei Hospital.

This work was supported by a Grant-in-Aid for Research on Hepatitis and BSE from Ministry of Health Labour and Welfare of Japan, and Scientific Research from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1 Hayashi N, Takehara T. Antiviral therapy for chronic hepatitis C: past, present, and future. *J Gastroenterol* 2006; 41: 17–27.
2 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–965.
3 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.
4 Hadziyannis SJ, Sette H Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; 140: 346–355.
5 Zeuzem S, Hultcrantz R, Bourliere M *et al.* Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004; 40: 993–999.
6 Hiramatsu N, Hayashi N, Kasahara A *et al.* Improvement of liver fibrosis in chronic hepatitis C patients treated with natural interferon alpha. *J Hepatol* 1995; 22: 135–142.
7 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; 27: 1394–1402.
8 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; 29: 1124–1130.
9 Kasahara A, Tanaka H, Okanoue T *et al.* Interferon treatment improves survival in chronic hepatitis C patients showing biochemical as well as virological responses by preventing liver-related death. *J Viral Hepatitis* 2004; 11: 148–156.
10 Imai Y, Kasahara A, Tanaka H *et al.* Interferon therapy for aged patients with chronic hepatitis C: improved survival in patients exhibiting a biochemical response. *J Gastroenterol* 2004; 39: 1069–1077.
11 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; 38: 645–652.
12 McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; 123: 1061–1069.
13 Shiffman ML, Ghany MG, Morgan TR *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; 132: 103–112.
14 Reddy KR, Shiffman ML, Morgan TR *et al.* Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007; 5: 124–129.
15 Shiffman ML, Salvatore J, Hubbard S *et al.* Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology* 2007; 46: 371–379.
16 Paterson DL, Swindells S, Mohr J *et al.* Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Ann Intern Med* 2000; 133: 21–30.
17 Strader DB, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 39: 1147–1171.
18 Dienstag JL, McHutchison JG. American Gastroenterological Association medical position statement on the management of hepatitis C. *Gastroenterology* 2006; 130: 225–230.
19 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; 352: 1426–1432.
20 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1485–1492.
21 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; 339: 1493–1499.

22 Hiramatsu N, Kasahara A, Nakanishi F *et al.* The significance of interferon and ribavirin combination therapy followed by interferon monotherapy for patients with chronic hepatitis C in Japan. *Hepatol Res* 2004; 29: 142–147.
23 Bronowicki JP, Ouzan D, Asselah T *et al.* Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. *Gastroenterology* 2006; 131: 1040–1048.
24 Hiramatsu N, Oze T, Yakushijin T, *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2b plus ribavirin. *J Viral Hepat* 2009; In press.

Journal of Viral Hepatitis, 2009, 16, 346–351 doi:10.1111/j.1365-2893.2009.01099.x

Impact of early viral kinetics on pegylated interferon alpha 2b plus ribavirin therapy in Japanese patients with genotype 2 chronic hepatitis C

H. Nomura,[1] Y. Miyagi,[1] H. Tanimoto[1] and H. Ishibashi[2] [1]*The Center for Liver Diseases, Shin-Kokura Hospital, Kitakyushu, Japan; and* [2]*Clinical Research Center, National Nagasaki Medical Center, Nagasaki, Japan*

Received July 2008; accepted for publication October 2008

SUMMARY. The recommended therapy for genotype-2 chronic hepatitis C is a regimen of pegylated interferon alpha (peginterferon) plus ribavirin. This study was conducted to determine the value of early viral kinetics as a predictive factor for sustained virologic responder (SVR). Peginterferon alpha 2b (1.5 μg/kg/week) plus weight-based ribavirin (600–1000 mg/day) was administered to 51 patients with chronic HCV genotype 2 for 24 weeks. The HCV-RNA loads were measured at the baseline, hour 24, and week 1. The rebound index (RI, an index obtained from the viral load of week 1 divided by that of hour 24) was calculated. Compared with the baseline, the viral load at hour 24 for SVR was reduced by more than 1-log: it continued to decline thereafter, and at week 1 it was significantly lower than at hour 24 ($P < 0.05$). The viral load for non-SVR increased again between hour 24 and week 1. The SVR of patients with RI ≤1.0 was 100% (39/39). The SVR conversion for rapid virologic responders was 92% (35/38). The RI (≤1.0) was the only significant independent factor for SVR by multiple logistic regression analysis and is the first predictive factor in 24-week peginterferon plus ribavirin therapy for patients infected with genotype 2.

Keywords: chronic hepatitis C, early viral kinetics, genotype 2, pegylated interferon plus ribavirin, rebound index.

INTRODUCTION

The pegylated interferon alpha 2b (peginterferon) plus ribavirin combination therapy is recommended to treat genotype 2 chronic hepatitis C [1,2]. The two major predictive factors for a sustained virologic response (SVR) to interferon therapy are hepatitis C virus (HCV) genotype and viral load [3–7]. In Japan, the major genotypes include types 1 and 2 [8]. Compared with the former, the therapeutic efficacy of IFN is higher with the latter [8,9]. The duration of peginterferon plus ribavirin therapy for chronic hepatitis C is defined as 48 weeks for genotype 1 and 24 weeks for genotype 2 [10,11]. Attempts have been made to shorten the duration of the peginterferon plus ribavirin therapy for genotype 2 from 24 weeks to 12 or 16 weeks for rapid virologic responders (RVR; undetectable HCV-RNA at week 4) [12–18]. When peginterferon plus ribavirin is administered for 24 weeks, the rate of SVR is about 80% with relapse occurring in about 20%. It is believed that RVR is the primary predictive factor for SVR in the treatment of peginterferon plus ribavirin for genotype 2.

This study focused on early viral kinetics and RVR as predictive factors for SVR in the treatment of HCV patients with genotype 2 with peginterferon plus ribavirin. It was determined that rebound index (RI), a new index computed from early viral kinetics, is the first predictive factor for SVR and a substitute for RVR.

Abbreviations: EVR, early virologic response; NVR, nonvirologic response; RI, rebound index; RVR, rapid virologic response; SVR, sustained virologic responder.

Correspondence: Hideyuki Nomura, MD, The Center for Liver Diseases, Shin-Kokura Hospital, 1-3-1, Kanada, Kokurakita-ku, Kitakyushu, Fukuoka 803-8505, Japan.
E-mail: h-nomura@shin-kokura.gr.jp

PATIENTS AND METHODS

Chronic HCV genotype 2 infected patients were eligible for enrollment if they fulfilled the following pretreatment criteria: baseline elevated serum alanine aminotransferase (ALT) levels, detectable serum HCV RNA via nucleic acid testing, HCV genotype 2, viral loads ≥5.30 log IU/mL, age ≥30 years, and a liver biopsy in the past 3 months consistent with chronic hepatitis (F1–F3) diagnosed based on the scoring system of Desmet *et al.* [19]. Fifty-one patients were treated with subcutaneous peginterferon alpha 2b (1.5 μg/kg/week) (PegIntron; Schering-Plough, Osaka, Japan) and

oral ribavirin (600 mg/day based on weight: <60 kg, 800 mg; 60–80 kg, 1000 mg; >80 kg) (Rebetol; Schering-Plough) for 24 weeks.

The 51 patients who participated in this study consisted of 28 males and 23 females ranging in age from 30 to 71 years, with a mean age of 52.1 years. Treatment was interrupted in three patients due to the development of adverse events. The remaining 48 patients completed 24 weeks of treatment. For all 48 patients, the total dosage of peginterferon or ribavirin exceeded 80% of the planned total dosage.

Peginterferon was administered at 9:00 in the morning at the initial, second, and third dosing points. The HCV loads were tested immediately before the start of treatment, at hour 24, and in weeks 1 and 2. The coefficient derived by dividing the viral load of week 1 by that of hour 24 was defined as the RI. The patients were grouped into the following 3 groups based on the RI and viral load in week 1: group A, RI >1.0; group B, RI ≤1.0 and viral load ≥3.70 log IU/mL in week 1; group C, RI ≤1.0 and viral load <3.70 log IU/mL in week 1.

The qualitative test for HCV-RNA was conducted five times (at weeks 4, 8, and 12, at the completion of treatment, and at week 24 after the completion of therapy). Patients showing the absence of HCV-RNA by week four were designated as RVR; and those with viral negativity between weeks 5 and 12, early virologic responders (EVR). The patients who remained HCV-RNA-negative until week 24 after the therapy was completed were defined as SVR and all other patients were designated non-SVR. Those who failed to achieve HCV-RNA negativity by the end of the treatment were designated nonvirologic responders (NVR).

Frozen sera were collected from the patients before and during IFN treatment, and the viral loads were measured by employing a quantitative HCV-RNA PCR assay (COBAS Amplicor HCV Monitor Test version 2.0 using a 10-fold dilution method, Roche Diagnosis, Tokyo, Japan), which has a lower threshold of quantification of 3.70 log IU/mL and an outer limit of quantification of 6.71 log IU/mL. A quantitative test for serum HCV-RNA was performed by using an Amplicor-HCV kit version 2.0 (Roche Diagnosis) and the results were labelled as positive or negative. The lower limit of detection was 1.70 log IU/mL. The preserved serum that produced a negative result for qualitative analysis of HCV-RNA was later re-examined by using the COBAS TaqMan HCV (AUTO) (Roche Diagnosis). If both tests produced negative results, the sample was judged to be HCV-RNA-negative. All testing was performed at a single reference laboratory. The HCV genotype was determined by a type-specific primer from the core region of the HCV genome. The protocol for genotyping was carried out as previously described.

The criteria for exclusion were: (i) clinical or biochemical evidence of hepatic decomposition; and advanced cirrhosis identified by ascites, encephalopathy, or hepatocellular carcinoma; (ii) white blood cell count of less than 3000/mm^3 and platelet count of less than 50 000/mm^3; (iii) concomitant liver disease other than hepatitis C (hepatitis B surface antigen- or human immunodeficiency virus-positive); (iv) excessive active alcohol consumption exceeding 60 g/day or drug abuse; (v) severe psychiatric disease; and (vi) antiviral or corticosteroid therapy within the 12 months prior to enrollment. Both peginterferon alpha-2b and ribavirin were discontinued if the haemoglobin level, white blood cell count, or platelet count fell below 8.5 g/dL, 1000/mm^3 and 25 000/mm^3, respectively. The treatment was also discontinued if severe general fatigue, hyperthyroidism, interstitial pneumonia or severe haemolytic problems developed, continuation of treatment was judged not to be possible by the attending physician, or the patient no longer desired to continue treatment.

This study was conducted at the Shin-Kokura Hospital between December 2004 and June 2007. The study protocol was approved by the institutional ethics committee of Shin-Kokura Hospital and all patients gave informed consent to participate in this study, which was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Sustained virologic responder was analysed on an intention-to-treat basis. Differences between viral loads among groups were analysed using the Student's *t*-test and Mann–Whitney rank-sum test. Multivariate logistic regression analysis was used to determine predictive factors for SVR. We also calculated odds ratios and 95% confidence intervals. Predictive factors associated with SVR included: age, sex, body mass index (BMI), HCV-RNA loads, ALT levels, platelet counts, haemoglobin levels, RI, and the time HCV-RNA became undetectable.

All statistical analyses were conducted on a Macintosh computer using STATVIEW 5.0 (Abacus Concepts, Berkeley, CA, USA). Values for $P < 0.05$ were considered to be statistically significant.

RESULTS

Patient population

Of 51 patients, 48 patients completed the 24-week regimen. Of these 48 patients, 40 achieved SVR, resulting in an SVR rate of 83.3% (40/48). Seven patients remained HCV-RNA negative until week 24 of treatment but became positive again after the completion of the treatment. One patient failed to achieve HCV-RNA negativity by the end of the treatment. Of the three patients who interrupted treatment, two patients dropped out in weeks 17 and 19 due to general malaise and the other patient suffered from systemic eczema in week 5, necessitating the interruption of medication. Two of these three patients were SVR and one was non-SVR. The intention to treat analysis yielded a figure of 82.4% (42/51).

Table 1 Baseline characteristics of patients by SVR and non-SVR

	SVR $n = 42$	Non-SVR $n = 9$	Total $n = 51$
Age (years)	50.8 (12.7)	57.5 (12.7)	51.7 (12.6)
Male (%)	23 (55%)	5 (55%)	28 (55%)
Laboratory			
ALT (IU/L)	115 (111)*	56 (16)*	108 (106)
Haemoglobin (g/dL)	14.6 (1.6)	15.1 (1.3)	14.6 (1.5)
Platelet count ($\times 10^4/mm^3$)	19.1 (6.3)†	16.2 (6.2)†	18.7 (6.2)
HCV RNA loads (log IU/mL)	5.95 (0.47)‡	6.45 (0.33)‡	6.01 (0.48)
BMI (kg/m^2)	22.7 (2.8)	22.2 (3.6)	22.6 (2.8)

Values represent means with standard deviation in parentheses or as absolute values with percentages in parentheses.
*$P < 0.01$ for SVR *vs* non-SVR.
†$P < 0.001$ for SVR *vs* non-SVR.
‡$P < 0.05$ for SVR *vs* non-SVR.
SVR, sustained virologic responder; ALT, alanine aminotransferase; BMI, body mass index.

The baseline characteristics of these 51 patients by SVR and non-SVR are shown in Table 1. The mean age was not significantly different between SVR at 50.8 years and non-SVR at 57.4 years. The ALT level and platelet counts were significantly higher ($P < 0.01$ and $P < 0.001$, respectively) while the HCV-RNA load was significantly lower ($P < 0.05$) in patients with SVR.

Early viral kinetics and rebound index in relation to SVR and non-SVR

Early viral kinetics and the RI in relation to SVR and non-SVR are shown in Table 2 and Fig. 1. HCV-RNA load for all SVR patients was reduced by 1-log in hour 24. The viral load thereafter (in week 1) was significantly reduced in contrast to that of hour 24 ($P < 0.05$). Furthermore, in week 2, the viral load was significantly reduced compared with that of week 1 ($P < 0.001$). With the exception of one patient, none of the patients with SVR showed a rise in the viral load in week 1. Compared with the baseline, the viral load in week 1 was reduced by more than 1-log in all SVR patients. The viral load for non-SVR was reduced by 1-log in hour 24 but a 1-log reduction was not achieved with NVR. Thereafter, the viral load rose again in week 1, then was reduced in week 2. The viral loads of all nine patients exhibited an increase in week 1, and the viral load of three of these patients in week 1 failed to be reduced from the baseline by 1-log. Among these three patients, HCV-RNA became negative in week 12 in two patients but reverted to positive 1 month after the completion of the treatment. The RI (0.43) of SVR patients was significantly lower than that of non-SVR (4.13) ($P < 0.05$).

Table 2 Kinetics of HCV RNA and RI during the first 2 weeks of treatment relative to SVR and non-SVR.

	SVR ($n = 42$)	Non-SVR ($n = 9$)	Total ($n = 51$)
HCV loads (log IU/mL)			
Before	5.95 (0.47)*	6.45 (0.33)*	6.01 (0.48)
Hour 24	4.56 (0.75)†	4.97 (1.23)	4.68 (0.86)
Week 1	4.02 (0.69)†,‡	5.49 (0.90)	4.30 (0.95)
Week 2	3.77 (0.45)‡	4.14 (1.15)	3.97 (0.85)
RI	0.63 (0.09)*	2.13 (0.33)*	0.92 (0.12)

Values represent means with standard deviation in parentheses. SVR, sustained virologic responder; SD, standard deviation.
*$P < 0.05$ for SVR *vs* non-SVR.
†$P < 0.05$ for hour 24 *vs* week 1 in SVR.
‡$P < 0.001$ for week 1 *vs* week 2 in SVR.

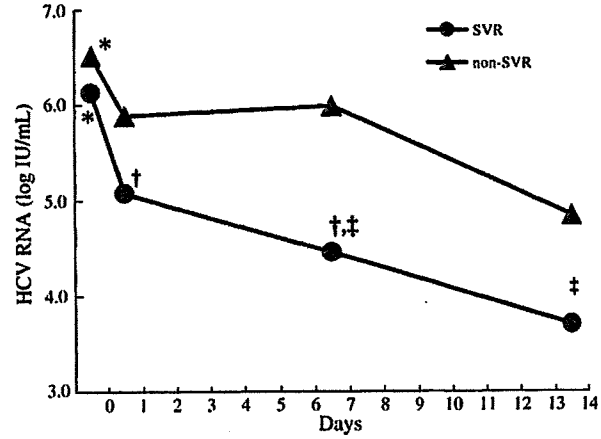

Fig. 1 Kinetics of HCV-RNA during the first 2 weeks of therapy relative to SVR (black circle) and non-SVR (black triangle). *$P < 0.05$ for SVR *vs* non-SVR, †$P < 0.05$ for hour 24 *vs* week 1 in SVR, ‡$P < 0.001$ for week 1 *vs* week 2 in SVR. SVR, sustained virologic response.

SVR and non-SVR in relation to the timing of HCV-RNA negativity in groups A, B and C

Sustained virologic responder and non-SVR for groups A, B and C stratified by the timing of HCV-RNA negativity are shown in Table 3. Among the 48 patients, there were 38 RVR (79.2%), 9 EVR (18.7%), and 1 NVR (2.1%). The percentages for achieving SVR by RVR, EVR, and NVR were 92.1%, 55.6%, and 0.0%, respectively. The percentages of achieving SVR in groups A, B and C were 11%, 100%, and 100%, respectively. In groups B and C with the

Table 3 SVR and non-SVR in relation to the timing of HCV-RNA negativity in group A, B and C

	RVR $n = 38$	EVR $n = 9$	NVR $n = 1$	Total $n = 48$
RI>1.0 (Group A, $n = 9$)				
SVR (%)	1 (25)	0 (0)	0 (0)	1 (11)
Non-SVR (%)	3 (75)	4 (100)	1 (100)	8 (89)
RI ≤1.0, ≥3.7 log IU/mL* (Group B, $n = 23$)				
SVR (%)	18 (100)	5 (100)	0 (0)	23 (100)
Non-SVR (%)	0 (0)	0 (0)	0 (0)	0 (0)
RI ≤1.0, <3.7 log IU/mL* (Group C, $n = 16$)				
SVR (%)	16 (100)	0 (0)	0 (0)	16 (100)
Non-SVR (%)	0 (0)	0 (0)	0 (0)	0 (0)
Total ($n = 48$)				
SVR (%)	35 (92)	5 (56)	0 (0)	40 (83)
Non-SVR (%)	3 (8)	4 (44)	1 (100)	8 (17)

*HCV RNA loads at week 1.
SVR, sustained virologic responder; RVR, rapid virologic responder; EVR, early virologic responder; NVR, non-virologic responder; RI, rebound index.

RI below 1.0, all became HCV-RNA-negative within 8 weeks, thus achieving SVR status. The 24-week peginterferon plus ribavirin treatment for genotype 2 required the RI to be less than 1.0. Among the patients with a RI of ≤1.0, 16 had a viral load of less than 3.7 log IU/mL (Group C) at week 1. These patients were considered to be super-high responders to peginterferon. The early viral kinetics of these patients are shown in Table 4. The group included nine males and seven females with a mean age of 47.1 years. The mean age for men was 50.6 years, which was higher than 42.8 years for women but the difference was not statistically significant. The viral load of these 16 patients before treatment was 5.90 log IU/mL, which was significantly lower than 6.22 log IU/mL, viral load for other SVR ($P < 0.01$). Of these 16, the viral load up to hour 24 was less than 3.70 log IU/mL in six patients. HCV-RNA was negative in week 2 in five of these six patients.

The viral loads of SVR patients in group A at baseline (RI >1.0), hour 24, weeks 1 and 2 were 6.08, 3.95, 4.40, and <3.70 log IU/mL, respectively. Compared with the viral load immediately before treatment, that at hour 24 was reduced by more than 2-log10.

Three patients interrupted treatment

Among the 51 patients who participated in the study, treatment was interrupted in three due to the development of adverse effects. These patients dropped out in weeks 5, 17, and 19. In these three patients, HCV-RNA became negative in week 4 and their RI was below 1.0. The patient who was discontinued in week 5 showed a relapse of HCV-RNA during a subsequent observation. The viral load for the two patients who dropped out in weeks 17 and 19 was less than 3.70 log IU/mL in week 1, and HCV-RNA continued to be negative 24 weeks after drug withdrawal. These two patients were judged to be SVR.

Predictive factors of SVR by multivariate analysis

Rebound index (≤1.0) was the only significant independent factor for SVR by multiple logistic regression analysis (Table 5). All other factors were not significant.

Table 4 Early viral kinetics of patients in super-high responder group (Group C)

			HCV loads (log IU/mL)			HCV-RNA	
Number	Age	Sex	Before	Hour 24	Week 1	Week 2	Week4
1	30	M	6.23	<3.70	<3.70	Negative	Negative
2	42	M	5.37	<3.70	<3.70	Negative	Negative
3	45	M	5.98	<3.70	<3.70	Negative	Negative
4	56	M	6.20	<3.70	<3.70	Negative	Negative
5	32	F	5.40	<3.70	<3.70	Negative	Negative
6	66	F	5.54	<3.70	<3.70	Positive	Negative
7	42	M	5.41	4.64	<3.70		Negative
8	48	M	6.08	4.28	<3.70		Negative
9	59	M	6.08	4.46	<3.70		Negative
10	66	M	6.28	4.23	<3.70		Negative
11	67	M	5.70	4.80	<3.70		Negative
12	30	F	6.18	4.41	<3.70		Negative
13	31	F	5.89	4.51	<3.70		Negative
14	32	F	5.94	4.08	<3.70		Negative
15	42	F	5.40	4.34	<3.70		Negative
16	67	F	5.70	4.63	<3.70		Negative

Table 5 Predictive factors of SVR by multivariate analysis

Factor	Category	Odds ratio	95% CI	*P*-value
Age	≥50 years	0.622	0.035–11.114	0.746
	<50 years	1		
Sex	Male	1.972	0.109–35.799	0.646
	Female	1		
BMI	<22.5	1.251	0.085–18.462	0.871
	≥22.5	1		
HCV load	<6.0 logIU/mL	0.98	0.061–15.788	0.988
	≥6.0 logIU/mL	1		
ALT	<50 IU/L	0.757	0.038–15.240	0.856
	≥50 IU/L	1		
Platelet count	≥18 × 10^4/mm^3	1.795	0.104–31.019	0.687
	<18× 10^4/mm^3	1		
Haemoglobin level	<14 mg/dL	0.398	0.012–12.7171	0.602
	≥14 mg/dL	1		
RI	≤1.0	689.586	4.214–>999.999	0.012
	>1.0	1		
Time to HCV RNA negativity(–)	≤Week 4	1.612	0.050–51.632	0.787
	>Week4	1		

BMI, body mass index; ALT, alanine aminotransferase.

DISCUSSION

The early viral kinetics in association with the peginterferon plus ribavirin treatment for genotype 1 have been reported [20,21]; but reports on early viral kinetics are scarce when the same combination is applied to genotype 2. This is the first investigation of early viral kinetics during peginterferon plus ribavirin therapy for genotype 2 chronic hepatitis C patients with high viral loads. We found that the RI (a new index) that is computed from the early viral kinetics is the first predictive factor for SVR as a substitute for RVR as a result of multiple analysis data. Patients with a RI of less than 1.0 and a viral load of less than 3.7 log IU/mL in week 1 were also identified as super-high responders to peginterferon plus ribavirin therapy.

The serum concentration of peginterferon alpha 2b peaked around 24 h, followed by a gradual decrease thereafter [22,23]. Thus the earlier studies on viral kinetics in association with peginterferon plus ribavirin for genotype 1 reported that the HCV load declines in hour 24 and increases again in week 1 [20,21]. In the responder group, the HCV load continues to decline every week thereafter [21]. A similar pattern is also seen in peginterferon monotherapy [22]. However, there are few reports on early viral kinetics involving genotype 2. In this study, the early viral kinetics of genotype 2 was investigated. Noting this increase in week 1, the viral load in week 1 was divided by that of hour 24 and the resultant coefficient was defined as the RI. In this study, the SVR rate was 100% for groups B and C, with the RI being less than 1.0. In these groups, HCV-RNA was eliminated by week 12 in all patients. On the other hand, re-emergence of virus was noted in 8% among the RVR. These findings suggested that the RI is the first predictive factor for SVR as a substitute for RVR in 24-week peginterferon plus ribavirin therapy for genotype 2. For those patients with a RI of >1.0, treatment lasting more than 24 weeks appeared necessary.

Peginterferon plus ribavirin therapy results in SVR exceeding 80% in genotype 2 patients when treatment lasts for 24 weeks [1,2,9]. Because these patients are high responders to peginterferon plus ribavirin therapy, attempts have been made to shorten the duration of treatment [12–18]. Earlier, RVR patients have been treated for shorter periods (e.g. 12, 14 and 16 weeks) and it was reported that there was no difference in the SVR rate compared with the treatment duration of 24 weeks [12–15] According to a recent randomized study, the SVR rate is high even in RVR patients when treated for 24 weeks [16]. It has been reported that shortening of the treatment period results in economic advantages and reductions in the development of side effects [17]. Thus it becomes necessary to evaluate the super-high responder group to peginterferon plus ribavirin therapy who do not show reductions in the SVR rate even when the duration of treatment is reduced. Among those with genotype 2, about 80% or more convert to RVR but RVR alone does not sufficiently explain the state of super-high responders. In interferon therapy of genotype 2 patients, peginterferon alone produces therapeutic effects [2]. An HCV load below 3.0 log IU/mL on day 7 and undetectable HCV-RNA on day 29 were predictive of successful short-term treatment [18]. It is essential to identify super-high responders to peginterferon by using the early viral kinetics during the first 2 weeks of therapy. In this study, the viral load was investigated in week 1 following the

start of therapy, probably before the therapeutic effect of ribavirin manifests. Among 38 patients with RVR, 16 (group C) were found to have a viral load of less than 3.7 log IU/mL in week 1. It was believed that these patients constitute super-high responders to peginterferon; and that a high SVR rate may be reached when the duration of the peginterferon plus ribavirin therapy is curtailed to less than 12 weeks. The viral load of the two patients who had discontinued treatment in weeks 17 and 19 was less than 3.7 log IU/mL in week 1, and both converted to SVR after discontinuation of treatment. Further studies on a larger scale are needed.

CONCLUSION

Rapid virologic responder is a predictive factor for SVR in peginterferon plus ribavirin therapy. However, it was proven that the RI that was computed from the early viral kinetics in this study is the first predictive factor for SVR as a substitute for RVR by multiple logistic regression analysis. Patients with a RI less than 1.0 and a viral load of less than 3.7 log IU/mL (below the detectable level) in week 1 are also considered to be super-high responders to peginterferon plus ribavirin, thus constituting a group for whom the treatment period may be shortened. Further studies on a larger scale are necessary.

REFERENCES

1 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; 358: 958–965.

2 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.

3 Tsubota A, Chayama K, Ikeda K *et al.* Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. *Hepatology* 1994; 19: 1088–1094.

4 Reichard O, Glaumann H, Fryden A *et al.* Two-year biochemical, virological, and histological follow-up in patients with chronic hepatitis C responding in a sustained fashion to interferon alfa-2b treatment. *Hepatology* 1995; 21: 918–922.

5 Shiratori Y, Kato N, Yokosuka O *et al.* Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Tokyo-Chiba Hepatitis Research Group. *Gastroenterology* 1997; 113: 558–566.

6 Poynard T, Yuen MF, Ratziu V, Lai CL. Viral hepatitis C. *Lancet* 2003; 362: 2095–2100.

7 Dai CY, Chuang WL, Chang WY *et al.* Tumor necrosis factor-alpha promoter polymorphism at position-308 predicts response to combination therapy in hepatitis C virus infection. *J Infect Dis* 2006; 193: 98–101.

8 Nomura H, Kimura Y, Tada H *et al.* Predictive factors of a response to interferon therapy in chronic hepatitis C. *J Clin Gastroenterol* 1996; 23: 185–190.

9 Zeuzem S, Hultcrantz R, Bourliere M *et al.* Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004; 40: 993–999.

10 Hadziyannis SJ, Sette Jr H, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; 140: 346–355.

11 Strader DB, Wright T, Thomas DL, Seeff LB. American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; 39: 1147–1171.

12 Mangia A, Santoro R, Minerva N *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; 352: 2609–2617.

13 Dalgard O, Bjoro K, Hellum KB *et al.* Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. *Hepatology* 2004; 40: 1260–1265.

14 von Wagner M, Huber M, Berg T *et al.* Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; 129: 522–527.

15 Yu ML, Dai CY, Huang JF *et al.* A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; 56: 553–559.

16 Shiffman ML, Suter F, Bacon BR *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; 357: 124–134.

17 Dalgard O, Bjoro K, Ring-Larsen H *et al.* Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. *Hepatology* 2008; 47: 35–42.

18 Lagging M, Langeland N, Pedersen C *et al.* Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. *Hepatology* 2008; 47: 1837–1845.

19 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; 19: 1513–1520.

20 Izumi N, Asahina Y, Kurosaki M *et al.* A comparison of the exponential decay slope between PEG-IFN alfa-2b/ribavirin and IFN alfa-2b/ribavirin combination therapy in patients with chronic hepatitis C genotype 1b infection and a high viral load. *Intervirology* 2004; 47: 102–107.

21 Buti M, Sanchez-Avila F, Lurie Y *et al.* Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. *Hepatology* 2002; 35: 930–936.

22 Glue P, Rouzier-Panis R, Raffanel C *et al.* A dose-ranging study of pegylated interferon alfa-2b and ribavirin in chronic hepatitis C. *Hepatology* 2000; 32: 647–653.

23 Asahina Y, Izumi N, Umeda N *et al.* Pharmacokinetics and enhanced PKR response in patients with chronic hepatitis C treated with pegylated interferon alpha-2b and ribavirin. *J Viral Hepat* 2007; 14: 396–403.

J Gastroenterol (2009) 44:338–345
DOI 10.1007/s00535-009-0008-z

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Effective prediction of outcome of combination therapy with pegylated interferon alpha 2b plus ribavirin in Japanese patients with genotype-1 chronic hepatitis C using early viral kinetics and new indices

Hideyuki Nomura · Yugo Miyagi · Hironori Tanimoto · Masashi Higashi · Hiromi Ishibashi

Received: 6 August 2008 / Accepted: 19 October 2008 / Published online: 10 March 2009

Abstract
Background The rates of sustained virologic response (SVR) and relapse with pegylated interferon alpha 2b (peginterferon) plus ribavirin in patients with genotype-1 chronic hepatitis C (CHC) are approximately 50 and 30%, respectively. We investigated whether SVR and transient response (TR) can be differentiated during treatment using new indices calculated from early viral kinetics and the timing of when hepatitis C virus (HCV)-RNA becomes undetectable.
Methods Peginterferon alpha 2b (1.5 μg/kg per week) plus weight-based ribavirin (600–1,000 mg/day) were administered to 141 patients with genotype-1 CHC for 48 weeks. The HCV-RNA loads were measured at baseline, 24 h, week 1, and week 2. The rebound index (RI, viral load at week 1 divided by viral load at 24 h) and the second rebound index (RI-2nd, viral load at week 2 divided by viral load at 24 h) were calculated.
Results With SVR, the viral load was reduced at 24 h, did not rise during week 1 (RI $\leq$ 1.0), and was significantly reduced at week 2 ($P < 0.05$). Viral loads with TR and non-response increased at week 1. The SVR rate was 90% with RI $\leq$ 1.0, 96% with rapid viral responders, and 93% with RI-2nd <0.7 and week 8 early viral responders. The SVR rate with these 3 groups was 90% and administration for 48 weeks was recommended. With other groups, the SVR rate was 23% and the TR rate was 77%. Administration for 72 weeks was therefore recommended.
Conclusions We distinguished SVR from TR during treatment using two indices (RI and RI-2nd) and the timing of HCV-RNA negativity.

Keywords Chronic hepatitis C · Pegylated interferon plus ribavirin · Early viral kinetics · Rebound index · Genotype 1

Abbreviations

SVR	Sustained virologic response
TR	Transient response
NR	Non-response
RI	Rebound index
RI-2nd	Second rebound index
RVR	Rapid viral responder
W8EVR	Week 8 early viral response
W12EVR	Week 12 early viral response
LVR	Late viral responder
NVR	Non-viral responder

Introduction

The first choice of treatment of genotype 1 chronic hepatitis C (CHC) is combination therapy with pegylated interferon alpha 2b (peginterferon) and ribavirin. The duration of treatment for genotype 1 is 48 weeks [1, 2]. Factors predictive of sustained virologic response (SVR) to

H. Nomura (✉) · Y. Miyagi · H. Tanimoto
The Center for Liver Diseases, Shin-Kokura Hospital,
1-3-1 Kanada, Kokurakitaku, Kitakyushu,
Fukuoka 803-8505, Japan
e-mail: h-nomura@shin-kokura.gr.jp

M. Higashi
Department of Internal Medicine,
Higashi Hospital, Fukuoka, Japan

H. Ishibashi
Clinical Research Center,
National Nagasaki Medical Center, Nagasaki, Japan

peginterferon plus ribavirin include genotype, viral load, age, histology, and amino acid substitutions in the hepatitis C virus (HCV) [3–6]. None of these predictive factors is adequate in predicting SVR in patients with genotype 1 and a high viral load prior to treatment.

With the current standard of care of peginterferon plus ribavirin administered for 48 weeks, the SVR rate in patients with genotype 1 and a high viral load is about 50% [1, 2]. It has been recognized, however, that the SVR rate increases if the duration of treatment is extended to 72 weeks [7–9]. The result of treatment with peginterferon plus ribavirin is SVR, transient response (TR), or non-response (NR). At the end of treatment, about 80% of patients are HCV RNA-negative, but about 30% of these patients relapse (TR) after the end of treatment, resulting in an actual SVR rate of about 50%. To increase the SVR rate, this incidence of relapse must be reduced; to achieve this, the duration of treatment needs to be extended to 72 weeks for patients who may potentially relapse after the end of treatment [7]. Differentiation between SVR and TR is therefore essential during treatment.

HCV RNA negativity status at weeks 4 and 12 during treatment is important in predicting SVR [6, 10–12], with reduction in the SVR rate observed if HCV RNA is not undetectable by week 12. In other words, for the early determination of the therapeutic efficacy of peginterferon plus ribavirin treatment, HCV RNA negativity by week 4 (rapid viral responder: RVR), HCV RNA negativity by week 12 (early viral responder: EVR), and HCV RNA negativity by week 24 (late viral responder: LVR) are considered important. EVR is a better predictor of SVR than the predictive factors that can be determined prior to treatment. EVR is therefore considered to be an index of therapeutic effect in the early stage of peginterferon plus ribavirin treatment. In a recent trend, a duration of treatment of 72 weeks is being selected when HCV RNA is detected at week 12 but is undetectable at week 24. However, distinguishing SVR from TR during treatment is difficult by these two time points when HCV RNA is not detected.

For a more accurate determination of SVR and TR during treatment, HCV RNA was examined at week 8 in addition to weeks 4, 12, and 24, and the SVR rate was examined based on HCV RNA negativity at these time points. Early viral kinetics up to week 2, considered to be the index of the therapeutic effect of peginterferon alone, were also evaluated and two new indices were defined. Distinguishing SVR from TR during peginterferon treatment was possible by combining these new indices and the timing of HCV RNA negativity and allowed the assignment of patients to 48- or 72-week treatment as a result.

Patients and methods

A total of 149 patients with genotype 1 CHC were treated with peginterferon plus ribavirin at the Shin-Kokura Hospital between December 2004 and May 2006. Of these patients, treatment was interrupted in 8 patients, so this study was conducted on the remaining 141 patients who completed 48 weeks of treatment. Eighty were male and 61 were female, with ages ranging from 27 to 70 years (mean: 53.2 ± 10.8), and 109 individuals were naïve to interferon therapy. The viral load at enrollment exceeded 100,000 IU/ml. The results of liver biopsy conducted within 6 months of enrollment confirmed chronic hepatitis (F1-F3), and diagnosis was based on the scoring system of Desmet et al. [13]. All patients received 1.5 μg/kg of peginterferon alpha-2b (PegIntron, Schering-Plough, Osaka, Japan) administered subcutaneously once a week in combination with ribavirin (Rebetol, Schering-Plough, Osaka, Japan) administered orally at a daily dose of 600–1,000 mg based on body weight (600 mg for patients weighing less than 60 kg, 800 mg for those weighing 60–80 kg, and 1,000 mg for those weighing more than 80 kg).

Peginterferon was administered at 9:00 in the morning for the initial, second, and third doses. The HCV loads were measured immediately before the start of treatment, at 24 h post-dose, and at weeks 1 and 2. The coefficient derived by dividing the viral load at week 1 by that at 24 h was defined as the rebound index (RI), while the coefficient derived by dividing the viral load at week 2 by that at 24 h was called the second rebound index (RI-2nd). The patients were divided into the following 3 groups based on RI and RI-2nd: RI-A group (RI $\leq$ 1.0), RI-B group (RI $>$ 1.0 and RI-2nd $<$ 0.7), and RI-C group (RI $>$ 1.0 and RI-2nd $\geq$ 0.7).

The qualitative test for HCV RNA was conducted 6 times (at weeks 4, 8, 12, and 24, at the end of treatment, and at week 24 after the end of treatment). Patients who were HCV RNA-negative by week 4 were considered rapid viral responders (RVR), patients who were HCV RNA-negative between weeks 5 and 12 were considered early viral responders (EVR), and patients HCV RNA-negative between weeks 13 and 24 were considered late viral responders (LVR). EVR was further divided into week 8 EVR (HCV RNA-negative between weeks 5 and 8, W8EVR) and week 12 EVR (HCV RNA-negative between weeks 9 and 12, W12EVR). Patients HCV RNA-positive at week 24 were considered non-viral responders (NVR). Patients who remained HCV RNA-negative up to 24 weeks after the end of treatment were considered to have achieved SVR. Patients HCV RNA-negative by week 24 of treatment but who became positive again after the end of treatment were considered TR. Patients who failed to achieve HCV RNA negativity by the end of treatment were