Table 5. Factors associated with EVR among patients over 65 y.o.

Univariate analysis				
Factor		EVR	Non-EVR	*p* value
Number		52	93	
Age (y.o.)		67.9 ± 2.3	67.8 ± 2.5	0.66
Sex: male / female		28 / 24	27 / 66	0.003
White blood cells (/mm³)		5063 ± 1474	5001 ± 1422	0.76
Neutrophils (/mm³)		2566 ±1110	2551 ± 1071	0.87
Red blood cells (×10⁴/mm³)		426 ± 36	421 ± 38	0.64
Hemoglobin (g/dl)		13.7 ± 1.2	13.5 ± 1.2	0.21
Platelets (×10⁴/mm³)		16.5 ± 5.5	14.0 ± 4.6	0.009
AST (IU/L)		70 ± 51	70 ± 40	0.49
ALT (IU/L)		76 ± 58	70 ± 41	0.80
Serum HCV RNA (KIU/ml)*		1700	1900	0.62
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	25 / 10	47 / 20	0.54
	Activity, 0 - 1 / 2 - 3	16 / 19	29 / 37	0.52
Peg-IFN dose (μg/kg/week)‡		1.35 ± 0.24	1.25 ± 0.31	0.03
Ribavirin dose (mg/kg/day)‡		10.0 ± 2.2	9.6 ± 2.3	0.40

Multivariate analysis				
Factor	Category	Odds ratio	95% CI	*p* value
Sex	male / female	0.309	0.149 - 0.644	0.002
Platelets (×10⁴/mm³)	<12 / ≥12	-	-	N.S
Peg-IFN dose (μg/kg/week)‡	<1.2 / ≥1.2	2.481	1.079 - 5.705	0.03

*, Data shown are median values.
†, 43 Missing.
‡, Mean doses during 0 to 12 weeks.
N.S., not statistically significant.

Table 6. Factors associated with SVR among patients over 65 y.o.

Univariate analysis				
Factor		SVR	Non-SVR	*p* value
Number		45	100	
Age (y.o.)		68.0 ± 2.4	67.7 ± 2.5	0.45
Sex: male / female		27 / 18	28 / 72	<0.001
White blood cells (/mm³)		5006 ± 1516	5030 ± 1409	0.81
Neutrophils (/mm³)		2575 ±1130	2548 ± 1063	0.96
Red blood cells (×10⁴/mm³)		427 ± 40	421 ± 36	0.53
Hemoglobin (g/dl)		13.8 ± 1.3	13.5 ± 1.2	0.14
Platelets (×10⁴/mm³)		16.1 ± 5.6	14.3 ± 4.7	0.09
AST (IU/L)		71 ± 54	69 ± 40	0.47
ALT (IU/L)		76 ± 56	70 ± 43	0.77
Serum HCV RNA (KIU/ml)*		1700	2000	0.51
Histology (METAVIR)†	Fibrosis, 0 - 2 / 3 - 4	21 / 8	51 / 22	1.00
	Activity, 0 - 1 / 2 - 3	14 / 15	31 / 41	0.66
Peg-IFN dose (μg/kg/week)‡		1.27 ± 0.28	1.23 ± 0.33	0.31
Ribavirin dose (mg/kg/day)‡		8.8 ± 2.1	9.1 ± 2.5	0.38
Virologic response: EVR / non-EVR		41 / 4	11 / 89	<0.001

Multivariate analysis				
Factor	Category	Odds ratio	95% CI	*p* value
Sex	male / female	0.283	0.088 - 0.914	0.035
Virologic response	EVR / non-EVR	0.012	0.004 - 0.043	<0.001

*, Data shown are median values.
†, 43 Missing.
‡, Mean doses during treatment.

With respect to the side effects and discontinuance rate of treatment in aged patients with CH-C, treated with Peg-IFN plus ribavirin combination therapy, Reddy et al. reported that there was no difference related to the incidence and reason for side effects between non-aged and aged patients [6]. Another paper reported that the incidence of side effects was more frequent in aged patients [5]. In our study, not only the continuance rate without reduction of both drug decreased with age, but also the discontinuance rate of treatment increased with age, with a third of the patients over 70 y.o. discontinuing the treatment. The discrepancy, existing between our results and those reported in the former study cited above, is due to the difference in the number of aged patients enrolled; Reddy' s study analyzed a small cohort including only a few cases of patients over 65 y.o. and classified all those over 50 y.o. as aged patients.

Discontinuance of treatment due to progression of anemia was significantly higher in patients over 70 y.o., accounting for 43% (9/21) of the discontinuance in this group. Although the ratio of advanced fibrosis (score 3–4) increased with age, the high discontinuance rate due to anemia among patients over 70 y.o. was similar regardless of the progression of fibrosis (F0-2: <70 y.o., 1% (6/559) vs. ⩾70 y.o., 21% (6/28), p <0.0001; F3-4: <70 y.o., 0% (0/83) vs. ⩾70 y.o., 22% (2/9), p <0.0001). It is possible that poor hematopoietic function and renal function led to the progression of anemia in aged patients. For patients who develop severe anemia, using epoetin alpha or taribavirin, which are ribavirin prodrugs, has been shown to result in a lower incidence of anemia, although no significant increase of SVR has been reported so far, even with the addition of taribavirin to Peg-IFN [23–24].

With genotype 1 patients, the SVR rates were almost equal up to 65 y.o. (49–50%), but decreased to 31% (45/145) among the patients that were over 65 y.o., and even for those who completed the entire treatment schedule in this study. Since the degree of liver fibrosis and drug exposure have been shown to be associated with anti-viral efficacy, the progression of liver fibrosis or decrease of drug exposure with age could account for the reduction of SVR rate among the aged patients. However, the stratified analysis, according to the progression of liver fibrosis and drug exposure, revealed that older patients still yielded low a SVR rate (F0-2, Peg-IFN during the first 12 weeks ⩾1.2 μg/kg/week:<65 y.o., 55% (143/261) vs. ⩾65 y.o., 33% (15/46), p <0.0001; F0-2, Peg-IFN during the first 12 weeks <1.2 μg/kg/week:<65 y.o., 43% (26/60) vs. ⩾65 y.o., 23% (6/26), p = 0.07), which means that older patients would be difficult to treat. From our results showing a low SVR rate and a high discontinuance rate for patients over 65 y.o., the genotype 1 patients under 65 y.o were those who benefited the most from Peg-IFN plus ribavirin combination therapy. The high prevalence of treatment failure (non-SVR) among the aged patients seems to be due to the high populations of NR and LVR (Fig. 2). A high population of LVR is considered to lead to a higher transient response rate among aged patients, since those over 65 y.o. with LVR showed a much higher relapse rate (79%, 15/19) than those with EVR (21%, 11/52) (p <0.0001), as can be seen from Table 3.

In this study, multivariate analysis for SVR, in patients over 65 y.o., showed that the factors associated with SVR were EVR and gender. This indicates that better SVR can be expected even with older patients if EVR is attained and response-guided therapy guidelines can be useful for aged patients. A low SVR rate among aged female patients was as previously reported [7], although the mechanism remains unclear. This finding suggests that female patients should be treated before 65 y.o.

The next question is how aged patients should be treated in order to attain EVR. We have examined the impact of drug exposure on treatment efficacy [25–26] and reported that Peg-IFN is dose-dependently correlated with EVR [25]. In this study, the dose-dependent efficacy of Peg-IFN for EVR was also revealed in aged patients over 65 y.o., with less than 0.9 μg/kg/week of Peg-IFN leading to a low EVR rate for aged patients. If patients are difficult to treat with more than 1.2 μg/kg/week of Peg-IFN, using as much Peg-IFN as possible is desirable, in order to attain higher EVR rates. Accordingly, a reduction of Peg-IFN to 80% may need to be considered, although the manufacturer's drug information recommends reducing the dose of Peg-IFN to 50% of the assigned one. Since reduction of Peg-IFN has been reported to not affect the SVR rate after HCV RNA disappearance [26], using G-CSF for aged patients who develop severe neutropenia can be beneficial, especially in the first 12 weeks.

We also examined the negative prediction of SVR, i.e. an HCV RNA decrease at an earlier point of treatment than the usual prediction at treatment week 12 of a 2-log decrease, among aged patients with CH-C treated by Peg-IFN plus ribavirin combination therapy. We found that none of the patients without a 1-log decrease at week 4 or a 2-log decrease at week 8 could attain SVR, even if the complete treatment duration was given, the negative predictive value (NPV) for SVR equaled 100%. This earlier prediction is applied just as well to aged patients as to non-aged patients in order to avoid additional adverse effects. Recently, a genetic polymorphism near the *IL28B* gene has been reported to be associated with non-response to Peg-IFN plus ribavirin combination therapy [27–29], which is beneficial to patients. Nevertheless, even in the presence of this genetic polymorphism, NPV for SVR remains at 57–87%; 100% accuracy is not guaranteed. Thus, in addition to the pretreatment prediction, an earlier negative prediction for SVR during treatment is also considered to be useful.

We have shown in this study that, in the presence of genotype 2, HCV was easily eliminated even among aged patients; the SVR rates were over 75% for patients who had completed the treatment, and these rates were similar up to 70 y.o. The SVR rate of genotype 2 patients over 70 y.o. was 43%, however, the age limitation of the treatment among patients over 70 y.o. remains unclear, because of the small number of patients enrolled in this study. We have reported that the reduction of treatment drugs had little effect on anti-viral efficacy for patients with genotype 2, meaning that SVR can be attained even with aged patients who are usually given lower drug doses than non-aged patients [30]. Patients under 70 y.o. with genotype 2 should, at least, benefit from this therapy. The SVR rate was maintained among genotype 2 patients being 65–69 y.o., compared to genotype 1 patients. The higher efficacy with shorter treatment duration in genotype 2 aged patients can account for it.

In conclusion, the strategy of a response-guided therapy and an earlier negative prediction for SVR may be beneficial for aged patients, especially those with genotype 1. At present, aged patients up to 65–70 y.o. with CH-C can be candidates for Peg-IFN plus ribavirin combination therapy, if its efficacy and adverse effects are fully taken into account. At the same time, there is an urgent need to establish new treatment procedures, such as combination therapy with protease inhibitor plus polymerase inhibitor without Peg-IFN or ribavirin, for non-responders or patients

with poor tolerability for Peg-IFN plus ribavirin combination therapy among aged patients.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this paper.

Acknowledgments

Other institutions and participants in the Osaka Liver Forum are: Higashiosaka City Central Hospital, S. Iio; Osaka Medical Center for Cancer and Cardiovascular Diseases, K. Imanaka; Kinki Central Hospital of Mutual Aid Association of Public School Teachers, E. Hayashi; Sumitomo Hospital, A. Yamada; Itami City Hospital, T. Kitada; Otemae Hospital, Y. Doi; Suita Municipal Hospital, T. Nagase; NTT West Osaka Hospital, A. Kaneko; National Hospital Organization Minami Wakayama Medical Center, K. Fujimoto; Nishinomiya Municipal Central Hospital, H. Ogawa; Saiseikai Senri Hospital, K. Suzuki; Osaka Kaisei Hospital, N. Imaizumi; Kano General Hospital, S. Kubota; Saso Hospital, M. Nishiuchi; and Meiwa Hospital, Y. Hayakawa.
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] Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49:1335–1374.
[2] Hayashi N, Takehara T. Antiviral therapy for chronic hepatitis C: past, present, and future. J Gastroenterol 2006;41:17–27.
[3] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, 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.
[4] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.
[5] Antonucci G, Longo MA, Angeletti C, Vairo F, Oliva A, Comandini UV, et al. The effect of age on response to therapy with peginterferon alpha plus ribavirin in a cohort of patients with chronic HCV hepatitis including subjects older than 65 yr. Am J Gastroenterol 2007;102:1383–1391.
[6] Reddy KR, Messinger D, Popescu M, Hadziyannis SJ. Peginterferon alpha-2a (40 kDa) and ribavirin: comparable rates of sustained virological response in sub-sets of older and younger HCV genotype 1 patients. J Viral Hepat 2009;16:724–731.
[7] Sezaki H, Suzuki F, Kawamura Y, Yatsuji H, Hosaka T, Akuta N, et al. Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. Dig Dis Sci 2009;54:1317–1324.
[8] Hiramatsu N, Oze T, Tsuda N, Kurashige N, Koga K, Toyama T, et al. Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? Hepatol Res 2006;35:185–189.
[9] Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006;43:54–63.
[10] McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827–1838.
[11] Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839–1850.
[12] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;349:825–832.
[13] Taura N, Yatsuhashi H, Hamasaki K, Nakao K, Daikoku M, Ueki T, et al. Increasing hepatitis C virus-associated hepatocellular carcinoma mortality and aging: long term trends in Japan. Hepatol Res 2006;34:130–134.
[14] Hamada H, Yatsuhashi H, Yano K, Daikoku M, Arisawa K, Inoue O, et al. Impact of aging on the development of hepatocellular carcinoma in patients with posttransfusion chronic hepatitis C. Cancer 2002;95:331–339.
[15] Hiramatsu N, Hayashi N, Kasahara A, Hagiwara H, Takehara T, Haruna Y, et al. Improvement of liver fibrosis in chronic hepatitis C patients treated with natural interferon alpha. J Hepatol 1995;22:135–142.
[16] Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, 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.
[17] Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, 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.
[18] Imai Y, Tamura S, Tanaka H, Hiramatsu N, Kiso S, Doi Y, et al. Reduced risk of hepatocellular carcinoma after interferon therapy in aged patients with chronic hepatitis C is limited to sustained virological responders. J Viral Hepat 2010;17:185–191.
[19] Kurokawa M, Hiramatsu N, Oze T, Mochizuki K, Yakushijin T, Kurashige N, et al. Effect of interferon alpha-2b plus ribavirin therapy on incidence of hepatocellular carcinoma in patients with chronic hepatitis. Hepatol Res 2009;39:432–438.
[20] Kasahara A, Tanaka H, Okanoue T, Imai Y, Tsubouchi H, Yoshioka K, 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 Hepat 2004;11:148–156.
[21] Imai Y, Kasahara A, Tanaka H, Okanoue T, Hiramatsu N, Tsubouchi 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.
[22] Yoshizawa H. Trends of hepatitis virus carriers. Hepatol Res 2002;24: S28–S39.
[23] Afdhal NH, Dieterich DT, Pockros PJ, Schiff ER, Shiffman ML, Sulkowski MS, et al. Epoetin alfa maintains ribavirin dose in HCV-infected patients: a prospective, double-blind, randomized controlled study. Gastroenterology 2004;126:1302–1311.
[24] Benhamou Y, Afdhal NH, Nelson DR, Shiffman ML, Halliman DG, Heise J, et al. A phase III study of the safety and efficacy of viramidine versus ribavirin in treatment-naive patients with chronic hepatitis C: ViSER1 results. Hepatology 2009;50:717–726.
[25] Oze T, Hiramatsu N, Yakushijin T, Kurokawa M, Igura T, Mochizuki K, et al. 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. J Viral Hepat 2009;16:578–585.
[26] Hiramatsu N, Oze T, Yakushijin T, Inoue Y, Igura T, Mochizuki K, et al. Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. J Viral Hepat 2009;16:586–594.
[27] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801.
[28] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104.
[29] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
[30] Inoue Y, Hiramatsu N, Oze T, Yakushijin T, Mochizuki K, Hagiwara H, et al. Factors affecting efficacy in patients with genotype 2 chronic hepatitis C treated by pegylated interferon alpha-2b and ribavirin: reducing drug doses has no impact on rapid and sustained virological responses. J Viral Hepat 2009;17:336–344.

Journal of Medical Virology 83:419–427 (2011)

Amino Acid Substitution in the Core Protein has no Impact on Relapse in Hepatitis C Genotype 1 Patients Treated With Peginterferon and Ribavirin

Yuko Inoue,[1] Naoki Hiramatsu,[1]* Tsugiko Oze,[1] Takayuki Yakushijin,[1] Kiyoshi Mochizuki,[1] Kazuto Fukuda,[2] Eiji Mita,[3] Yoshimichi Haruna,[4] Atsuo Inoue,[4] Yasuharu Imai,[2] Atsushi Hosui,[1] Takuya Miyagi,[1] Yuichi Yoshida,[1] Tomohide Tatsumi,[1] Shinichi Kiso,[1] Tatsuya Kanto,[1] Akinori Kasahara,[1] Tetsuo Takehara,[1] and Norio Hayashi[5]

[1]*Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Suita, Japan*
[2]*Ikeda Municipal Hospital, Ikeda, Japan*
[3]*National Hospital Organization Osaka National Hospital, Osaka, Japan*
[4]*Osaka General Medical Center, Osaka, Japan*
[5]*Kansai Rousai Hospital, Amagasaki, Japan*

Previous reports demonstrated that amino acid (aa) substitutions in the hepatitis C virus (HCV) core protein are predictors of non-virological responses to pegylated interferon (Peg-IFN) and ribavirin combination therapy. The aim of this study was to investigate the impact of core aa substitutions on viral kinetics during the treatment and relapse after the treatment. The 187 patients with HCV genotype 1 enrolled in this study were categorized into four groups according to core aa substitution patterns: double-wild group (n = 92), Arg70/Leu91; 70-mutant group (n = 42), Gln70/Leu91; 91-mutant group (n = 31), Arg70/Met91; and double-mutant group (n = 22), Gln70/Met91. The relationship between the core aa substitutions and the virological response was examined. Multivariate logistic regression analyses showed that substitution at aa 70 was significantly associated with a poor virological response during the first 12 weeks (decline of <1 log from baseline at week 4, <2 log at week 12), and substitution at aa 91 was significantly associated with detectable HCV RNA at week 24. With respect to relapse, only the ribavirin exposure (odds ratio (OR), 0.77; 95% confidence interval (CI), 0.60–0.98) and HCV RNA disappearance between weeks 13 and 24 (OR, 23.69; 95% CI, 5.44–103.08) were associated independently with relapse, with no correlation being found with the core aa substitutions and relapse. In conclusion, the results showed that core aa substitutions can be strong predictive factors at pretreatment of the non-response, but not for relapse, for virological responders with HCV RNA disappearance during treatment. *J. Med. Virol. 83:419–427, 2011.*

KEY WORDS: amino acid substitution; core protein; hepatitis C virus; peginterferon and ribavirin combination therapy; relapse

INTRODUCTION

The current standard of care for chronic hepatitis C patients is combination therapy using pegylated interferon (Peg-IFN) and ribavirin [Anonymous, 2002; Strader et al., 2004; Dienstag and McHutchison, 2006]. However, the treatment outcome in response to this combination therapy among patients infected with hepatitis C virus (HCV) genotype 1 is still unsatisfactory and the chance of sustained virological response ranges from 42% to 52% [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. Therefore, tailoring treatment regimens for individual patients has become an important issue.

Grant sponsor: Ministry of Health Labor and Welfare of Japan (Research on Hepatitis and BSE); Grant sponsor: Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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

Accepted 20 September 2010

DOI 10.1002/jmv.21975

Published online in Wiley Online Library (wileyonlinelibrary.com).

Outcome of therapy is influenced by various factors. Some host factors, such as age, sex, body weight, insulin resistance, and liver fibrosis have been reported as pretreatment factors affecting virological response to this combination therapy [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004; Romero-Gomez et al., 2005]. Recently, several genome-wide association studies identified single nucleotide polymorphisms (SNPs) near the interleukin (IL)-28B gene, which encodes interferon (IFN) lambda-3, as associated with response to Peg-IFN plus ribavirin treatment among patients infected with HCV of European [Suppiah et al., 2009], African [Ge et al., 2009], and Asian ancestry [Tanaka et al., 2009]. These studies suggest that host genetic variants may be associated strongly with response to IFN-alpha-based therapy. However, the ethical problem to perform host genetic search for all patients remains, and the sustained virological response rate is only 48–69% in patients having favorable IL-28B genotype to this combination therapy [Thompson et al., 2010].

Response-guided therapy is a dynamic approach to management of chronic hepatitis C patients based on the virological response at weeks 4 and 12 of treatment. At present, it is regarded as an excellent strategy for optimizing the treatment duration for individual patients. Earlier HCV RNA disappearance has been shown to lead to a higher sustained virological response rate [Ferenci et al., 2005; Berg et al., 2006; McHutchison et al., 2009], while patients without an early virological response, defined as showing an at least 2 log decrease from the baseline of HCV RNA levels at week 12 is recommended for discontinuing the treatment under the current guidelines [Anonymous, 2002; Strader et al., 2004; Dienstag and McHutchison, 2006].

In addition to viral kinetics during treatment, other viral factors have also been reported to be associated with this combination therapy outcome [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004; Shirakawa et al., 2008]. Previous studies indicated that amino acid (aa) 70 and/or 91 substitutions in the HCV core protein were independent pretreatment predictors of null or weak response to this combination therapy in genotype 1 patients [Akuta et al., 2007b,c]. The HCV core protein has been reported to inhibit signal transducer and activator of transcription (STAT)-1 phosphorylation, and disrupt the normal IFN-stimulated transcriptional response to viral infection [Lin et al., 2006]. It is supposed that the HCV core region might be associated with resistance to IFN therapy involving the Janus activated kinase (Jak)-STAT signaling cascade [Blindenbacher et al., 2003; Bode et al., 2003; Melen et al., 2004; de Lucas et al., 2005]. Recently, Okanoue et al. [2009] have demonstrated that wild type of core aa 70 and 91 are important for positive prediction of the virological response. However, the impact of core aa substitutions on the extent of HCV RNA decline during the treatment or virological relapse after completion of treatment has not yet been investigated in detail. Approximately 30% of genotype 1 patients who become HCV RNA negative at the end of the treatment will experience relapse [Hadziyannis et al., 2004]. Being able to distinguish between end-of-treatment responders with a high probability of relapse and those with a low probability of relapse will be useful in reducing relapse rates and improving treatment outcome.

The aim of this study was to evaluate the impact of aa substitutions in the HCV core protein on viral kinetics and virological relapse in patients with HCV genotype 1 treated by Peg-IFN alpha-2b and ribavirin combination therapy.

PATIENTS AND METHODS

Patient Selection and Study Design

Patients considered to be eligible for this study were those who were infected with HCV genotype 1, had a viral load more than 10^5 IU/ml, had started Peg-IFN alpha-2b (Schering-Plough K.K. Tokyo, Japan) and ribavirin (Schering-Plough K.K.) combination therapy from December 2005 to June 2008 at Osaka University Hospital and three other medical institutions taking part in the Osaka Liver Forum, and had been examined with respect to the aa sequences at positions 70 and 91 in the HCV core protein with pretreatment serum samples. Patients with the following criteria were excluded: hepatitis B virus or human immunodeficiency virus co-infection; decompensated liver disease; severe cardiac, renal, hematological, or chronic pulmonary disease; poorly controlled psychiatric disease; poorly controlled diabetes; and immunologically mediated disease. As a result of screening at the institutions concerned, 187 patients with HCV genotype 1 were enrolled in this study. Liver biopsy had been performed within 12 months prior to the treatment, and histological results were classified according to the METAVIR scoring system [Bedossa and Poynard, 1996].

Written informed consent was obtained from each patient, and the study protocol was reviewed and approved according to the ethical guidelines of the 1975 Declaration of Helsinki by Institutional Review Boards at the respective sites.

Peg-IFN alpha-2b and ribavirin dosages were based on body weight according to the manufacturer's instructions: Peg-IFN alpha-2b was given subcutaneously weekly (45 kg or less, 60 μg/dose; 46–60 kg, 80 μg/dose; 61–75 kg, 100 μg/dose; 76–90 kg, 120 μg/dose; and 91 kg or more, 150 μg/dose), and ribavirin was given orally daily (60 kg or less, 600 mg/day; 61–80 kg, 800 mg/day; and 81 kg or more, 1,000 mg/day). The drug doses were also modified based on the manufacturer's instructions according to the severity of the adverse hematologic effects.

Detection of Amino Acid Substitutions in Core Region

The nucleotide sequence encoding aa 1–191 (the core protein of HCV) was analyzed by direct sequencing as described by Akuta et al. [2005, 2007b]. In brief, HCV

RNA was extracted from the serum samples and converted to cDNA and two nested rounds of polymerase chain reaction (PCR) were performed. Primers used in the PCR were as follows: the first PCR was performed using cc11 (sense, 5′-GCC ATA GTG GTC TGC GGA AC-3′) and e14 (antisense, 5′-GGA GCA GTC CTT CGT GAC ATG-3′) primers. The second PCR was performed using cc9 (sense, 5′-GCT AGC CGA GTA GTG TT-3′) and e14 (antisense) primers. All samples were denatured initially at 95°C for 15 min. The 35 cycles of amplification were set as follows: denaturation for 1 min at 94°C, annealing of primers for 2 min at 55°C, and extension for 3 min at 72°C with an additional 7 min for extension. Then 1 μl of the first PCR product was transferred to the second PCR reaction. The conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR Purification Kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing Kit (Perkin–Elmer, Tokyo, Japan). The obtained nucleotide and amino acid sequences were compared with the prototype sequence of genotype 1b HCV-J (GenBank Accession No. D90208) [Kato et al., 1990]. Wild types virus encoded arginine (Arg) and leucine (Leu) at aa 70 and 91, respectively, and the aa substitutions were glutamine (Gln) or histidine (His) at aa 70 and methionine (Met) at aa 91. If the intensities of the band were similar, the case was regarded as competitive. Two patterns of mutant and competitive were labeled as mutant. In this study, patients were categorized into four groups according to aa substitution patterns: double-wild group, Arg70/Leu91; 70-mutant group, Gln or His70/Leu91; 91-mutant group, Arg70/Met91; and double-mutant group, Gln or His70/Met91.

Virological Tests

Serum HCV RNA level was quantified by PCR assay (COBAS Amplicor HCV Monitor Test v2.0, Chugai-Roche Diagnostics, Tokyo, Japan), with a sensitivity limit of 5,000 IU/ml and a dynamic range from 5,000 to 5,000,000 IU/ml.

Serum HCV RNA was assessed by qualitative PCR assay (COBAS Amplicor HCV Test v2.0, Chugai-Roche Diagnostics), with a detection limit of 50 IU/ml.

Efficacy Assessments

Patients who achieved negative HCV RNA at week 12 were defined as having a complete early virological response. Patients who became HCV RNA negative between weeks 13 and 24 were defined as having a late virological response. According to the established guidelines, the treatment was considered to have failed if the patients showed an insufficient virological response at week 12 (a detectable HCV RNA and a decrease of <2 log from the baseline level) or at week 24 (a detectable HCV RNA), and therapy was discontinued. The end-of-treatment response was defined as undetectable HCV RNA at week 48. Patients with end-of-treatment response and undetectable HCV RNA 24 weeks after completion of therapy were defined as having sustained virological response. Relapse was defined as a case in which HCV RNA had been undetectable at the end-of-treatment, but detectable during the 24-week follow-up after the treatment.

Drug Exposure

The amounts of Peg-IFN alpha-2b and ribavirin actually taken by each patient during the treatment period 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 μg/kg/week and ribavirin as mg/kg/day.

Data Collection

The medical records were retrospectively reviewed and the factors necessary for this examination were extracted: age, sex, body weight, body mass index (BMI), basic laboratory assessments, liver histology, quantitative and qualitative HCV RNA, dose of Peg-IFN alpha-2b and ribavirin received at each administration, and the response to treatment.

Statistical Analysis

Continuous variables are reported as the mean with standard deviation (SD) or median level, while categorical variables are shown as the count and proportion. In univariate analysis, the Mann–Whitney *U*-test (between two groups) or Kruskal–Wallis test (among more than three groups) was used to analyze continuous variables, while chi-squared and Fisher's exact tests were used for analysis of categorical data. For all tests, two-sided *P* values were calculated, and the results were considered statistically significant if $P < 0.05$. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were subjected to multivariate logistic regression analysis. Stepwise and multivariate logistic regression models were used to explore the independent factors that could be used to predict a virological response. Statistical analysis was performed using the SPSS program for Windows, version 15.0J (SPSS, Chicago, IL).

RESULTS

Baseline Characteristics of Study Groups

The total study population was predominately male (55.6%), with a mean age of 56.2 years. The baseline characteristics of all patients and the four study groups according to core aa substitution patterns are shown in Table I. Mean age of patients in the double-mutant group was higher than the other three groups ($P = 0.003$). More patients in the double-wild group had

TABLE I. Baseline Demographic and Viral Characteristics of Patients

Characteristic	Total (n = 187)	Double-wild (n = 92)	70-Mutant (n = 42)	91-Mutant (n = 31)	Double-mutant (n = 22)	*P* value[a]
Age (years)	56.2 ± 9.3	55.7 ± 9.2	57.0 ± 9.8	52.4 ± 9.9	61.8 ± 4.7	0.003
Sex (male/female)	104/83	51/41	26/16	18/13	9/13	0.444
Body weight (kg)	60.9 ± 11.6	60.9 ± 11.7	62.2 ± 11.7	62.5 ± 13.2	56.0 ± 7.5	0.193
Body mass index (kg/m^2)	22.8 ± 3.1	22.8 ± 3.0	22.8 ± 3.1	23.1 ± 3.6	22.1 ± 2.4	0.627
Past IFN therapy (naïve/experienced)	118/69	45/47	34/8	20/11	19/3	<0.001
HCV RNA (×10^3 IU/ml)[b]	1,700	2,100	1,400	1,500	1,230	0.122
Fibrosis (0–2/3–4)[c]	105/29	56/11	22/6	14/7	13/5	0.366
Activity (0–1/2–3)[d]	83/50	42/24	18/10	11/10	12/6	0.771
White blood cell (×10^6/l)	4,980 ± 1,520	4,990 ± 1,420	5,180 ± 1,760	4,890 ± 1,430	4,660 ± 1,560	0.795
Red blood cell (×10^{12}/l)	4.34 ± 0.46	4.33 ± 0.46	4.41 ± 0.52	4.39 ± 0.42	4.18 ± 0.32	0.145
Hemoglobin (g/dl)	13.9 ± 1.4	13.9 ± 1.4	14.0 ± 1.7	14.2 ± 1.4	13.5 ± 1.1	0.253
Platelet (×10^9/l)	161 ± 54	167 ± 49	165 ± 65	154 ± 60	138 ± 30	0.067
ALT (IU/l)	74 ± 61	73 ± 67	79 ± 56	81 ± 64	57 ± 37	0.263
γ-GTP (IU/l)	62 ± 74	47 ± 54	81 ± 89	70 ± 93	78 ± 78	0.032

IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase.
[a]*P* value for comparison among double-wild, 70-mutant, 91-mutant, and double-mutant.
[b]Values expressed as median.
[c]Data for 53 patients are missing.
[d]Data for 54 patients are missing.

been treated previously for HCV infection ($P < 0.001$). Patients in the double-wild group had significantly lower gamma-glutamyl transpeptidase (γ-GTP) levels ($P = 0.032$).

Progress of Patients

The progress of patients in this study is shown in Figure 1. Of the 187 patients, 183 completed 4 weeks of treatment. Among them, 133 were assessed based on HCV RNA dynamics between baseline and week 4. Those completing 12 weeks of treatment totaled 181, of which 154 were assessed for HCV RNA dynamics between baseline and week 12. Those completing 24 weeks of treatment totaled 153, and all were assessed for HCV RNA quantitatively or qualitatively at week 24. Those completing 48 weeks of treatment totaled 114. These 114 patients and the 55 patients who had discontinued treatment because of treatment failure entered a follow-up period. Among these 169 patients, 164 completed 24 weeks follow-up and the sustained virological response (SVR) rate

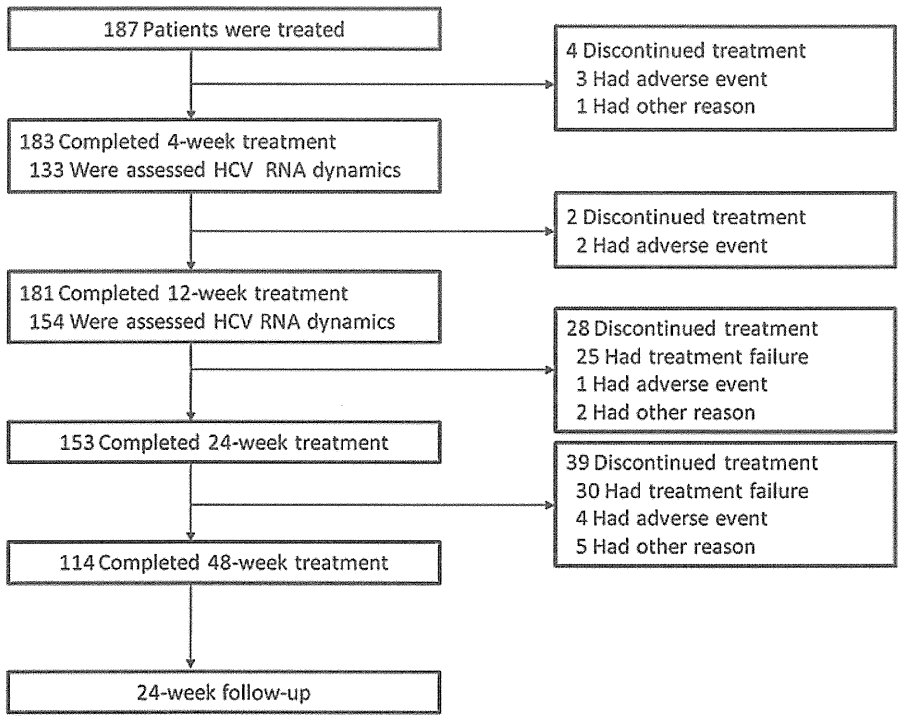

Fig. 1. Treatment and follow-up of the study patients. Treatment was discontinued for patients with <2 log decrease from the baseline HCV RNA level at week 12 or detectable HCV RNA at week 24.

TABLE II. Multivariate Analysis for Factors Associated With <1 log Decrease in HCV RNA Level at Week 4, <2 log Decrease at Week 12, Detectable HCV RNA at Week 24, and Relapse After Treatment

Factor	Category	Odds Ratio	95% CI	*P* value
HCV RNA <1 log decrease at week 4				
White blood cells ($\times 10^6$/l)	<5,000/5,000≤	—	—	NS
γ-GTP (IU/l)	<40/40≤	—	—	NS
Peg-IFN dose (μg/kg/week)	By 0.1 μg/kg/week	0.80	0.67–0.97	0.020
Core aa 70	Wild/mutant	1/2.80	1.16–6.75	0.022
HCV RNA <2 log decrease at week 12				
γ-GTP (IU/l)	<40/40≤	—	—	NS
Peg-IFN dose (μg/kg/week)	By 0.1 μg/kg/week	—	—	NS
Core aa 70	Wild/mutant	1/2.72	1.09–6.78	0.032
Detectable HCV RNA at week 24				
Platelet ($\times 10^9$/l)	<150/150≤	—	—	NS
γ-GTP (IU/l)	<40/40≤	1/2.46	1.02–5.95	0.045
Core aa 91	Wild/mutant	1/4.11	1.73–9.78	0.001
Relapse after treatment				
Ribavirin dose (mg/kg/day)	By 1 mg/kg/day	0.77	0.60–0.98	0.036
Virological response	Complete early virological response/late virological response	1/23.69	5.44–103.08	<0.001

CI, confidence interval; NS, not significant difference; γ-GTP, gamma-glutamyl transpeptidase; Peg-IFN, pegylated interferon; aa, amino acid.

was 48.2% (79/164), based on per-protocol set. Among the 106 patients who had an end-of-treatment response and completed follow-up, 27 showed relapse during the follow-up period; the relapse rate was 25.5% (27/106).

IMPACT OF CORE-RELAPSE AFTER TREATMENT (TABLE II)

Impact of core aa substitutions on <1 log viral decrease rate at week 4, <2 log at week 12, detectable HCV RNA at week 24, and virological relapse after treatment (Table II).

The impact of core aa substitutions on <1 log viral decrease rate at week 4, <2 log at week 12, detectable HCV RNA at week 24, and virological relapse after treatment (Table II).

The impact of the core aa substitutions on <1 log viral decrease at week 4, which is a predictor of non-sustained virological response; fewer than 5% of patients without 1 log decrease at week 4 had an sustained virological response [McHutchison et al., 2009] was examined. Among the 133 patients who completed 4 weeks of treatment, 31 failed to show a ≥1 log decrease of HCV RNA level at week 4. Univariate analysis for factors associated with <1 log decrease of HCV RNA level at week 4 was performed on the following variables: age, sex, body weight, BMI, history of past IFN therapy, baseline HCV RNA level, histological fibrosis and activity, white blood cell count, red blood cell count, hemoglobin level, platelet count, alanine aminotransferase (ALT) level, γ-GTP level, dose exposure of Peg-IFN and ribavirin, and aa substitutions in the HCV core protein. The results indicated that pretreatment white blood cell count, γ-GTP level, the mean dose of Peg-IFN during the first 4 weeks of treatment and single-spot substitution in the HCV RNA core position at aa 70 contributed to a <1 log decrease of HCV RNA level at week 4. Analysis of these factors by multivariate logistic regression analysis showed that substitution of aa 70 (odds ratio (OR) 2.80, 95% confidence interval (CI) 1.16–6.75, $P = 0.022$) as well as the mean dose of Peg-IFN (OR 0.80, 95% CI 0.67–0.97, $P = 0.020$) was independently associated with viral decline (<1 log) at week 4.

Next, the impact of the core aa substitutions on <2 log viral decrease rate at week 12, which is presently considered to be the most reliable predictor of non-sustained virological response [Fried et al., 2002; Davis et al., 2003] was examined. Among the 154 patients who completed 12 weeks of treatment, 25 failed to show a ≥2 log decrease of HCV RNA level at week 12. Univariate analysis was performed on the same factors in the preceding examination. As a result, pretreatment γ-GTP level, the mean dose of Peg-IFN during the first 12 weeks of treatment and single-spot substitution in the HCV RNA core position at aa 70 contributed to a <2 log decrease of the HCV RNA level. These factors were then analyzed by multivariate logistic regression analysis; only substitution of aa 70 (OR 2.72, 95% CI 1.09–6.78, $P = 0.032$) was found to be independently associated with an insufficient virological response (<2 log HCV RNA decrease from baseline level) at week 12.

The impact of the core aa substitutions on detectable HCV RNA at week 24, which is another non-sustained virological response predictor [Davis et al., 2003] was also examined. Among 153 patients who completed 24 weeks of treatment, 30 still had detectable HCV RNA at week 24. Univariate analysis revealed that pretreatment platelet count, γ-GTP level, and single-spot substitution in the HCV RNA core position at aa 91 contributed to the HCV RNA remaining positive. Multivariate logistic regression analysis, using these factors, indicated that substitution of aa 91 (OR 4.11, 95% CI 1.73–9.78, $P = 0.001$) as well as γ-GTP level (>40 IU/l) (OR 2.46, 95% CI 1.02–5.95, $P = 0.045$) was

independently associated with detectable HCV RNA at week 24.

Next, the factors associated with virological relapse after the treatment was examined. Univariate analysis was performed on the virological response (complete early virologival response or late virological response) in addition to the factors in the preceding examination, revealing the mean dose of ribavirin during the full treatment period and a late virological response, but not aa substitutions (single-spot substitution in the HCV RNA core position at aa 70, $P = 0.467$; aa 91, $P = 0.776$).

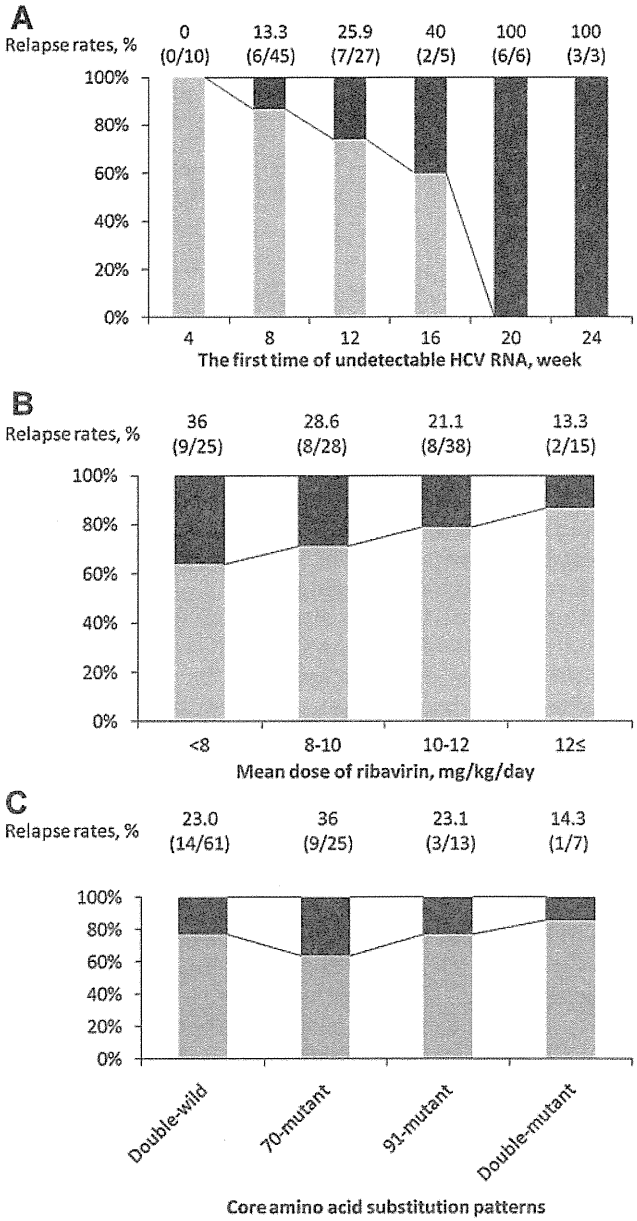

Fig. 2. Relapse rates according to the timing of HCV RNA disappearance (A), mean ribavirin dose (B), and core amino acid substitution patterns (C) in patients who had end-of-treatment response and completed 24-week follow-up. Relapse rates are shown as percentages and the number of patients with relapse in relation to the total number of patients examined is shown at the top of each column. Gray bar, sustained virological response; black bar, relapse.

These factors were analyzed by multivariate logistic regression analysis. This analysis revealed that the mean ribavirin dose (OR 0.77, 95% CI 0.60–0.98, $P = 0.036$) and a late virological response (OR 23.69, 95% CI 5.44–103.08, $P < 0.001$) were independently associated with relapse.

Relapse Rates According to the Timing of HCV RNA Disappearance, Ribavirin Dose, and Core aa Substitution Patterns

The relapse rates were indicated according to the time to the first non-detection of HCV RNA, mean ribavirin dose and core aa substitution patterns (Fig. 2). The relapse rate was 0% (0/10) in patients with undetectable HCV RNA during 1–4 weeks, and increased 13.3% (6/45) during 5–8 weeks, 25.9% (7/27) during 9–12 weeks, 40% (2/5) during 13–16 weeks, 100% (6/6) during 17–20 weeks, and 100% (3/3) during 21–24 weeks (Fig. 2A). Similarly, the relapse rates increased as the mean ribavirin dose decreased; 13.3% (2/15) in patients receiving ≥12 mg/kg/day of ribavirin, 21.1% (8/38) at 10–12 mg/kg/day, 28.6% (8/28) at 8–12 mg/kg/day, and 36% (9/25) at <8 mg/kg/day (Fig. 2B). On the other hand, the relapse rates were similar among the four core aa substitution patterns; 23.0% (14/61) in patients in the double-wild group, 36% (9/25) in 70-mutant group, 23.1% (3/13) in 91-mutant group, and 14.3% (1/7) in double-mutant group (Fig. 2C). In the subgroup of patients receiving <10 mg/kg/day of ribavirin, no significant difference of the relapse rates was observed between double-wild group and 70-mutant and/or 91-mutant group (31.3% (10/32) in double-wild group vs. 33.3% (7/21) in 70-mutant and/or 91-mutant group), and also in the patients receiving ≥10 mg/kg/day of ribavirin (13.8% (4/29) in double-wild group vs. 25% (6/24) in 70-mutant and/or 91-mutant group) (Fig. 3). Among patients with complete early virological response, the relapse rates were also similar between double-wild group and 70-mutant and/or 91-mutant group (13.7% (7/51) in double-wild vs. 18.4% (7/38) in 70-mutant and/or 91-mutant group). The impact of core aa substitutions on relapse rates in patients with late virological response could not be assessed because of the small number of patients.

DISCUSSION

Kobayashi et al. [2010] investigated the clinical and virological factors influencing these core aa substitutions in patients infected with HCV genotype 1 who had not received antiviral therapy, and found that HCV variants with wild type of core aa 70 and 91 significantly decreased with age, while those with the mutant type of core aa 70 and/or 91 significantly increased with age. Furthermore, they demonstrated that the proportion of patients with the mutant type of core aa 70 HCV variant significantly increased with an elevated γ-GTP level and a decrease in platelet counts. In this study, the significant differences of baseline demographics between patient groups according to core aa substitution pat-

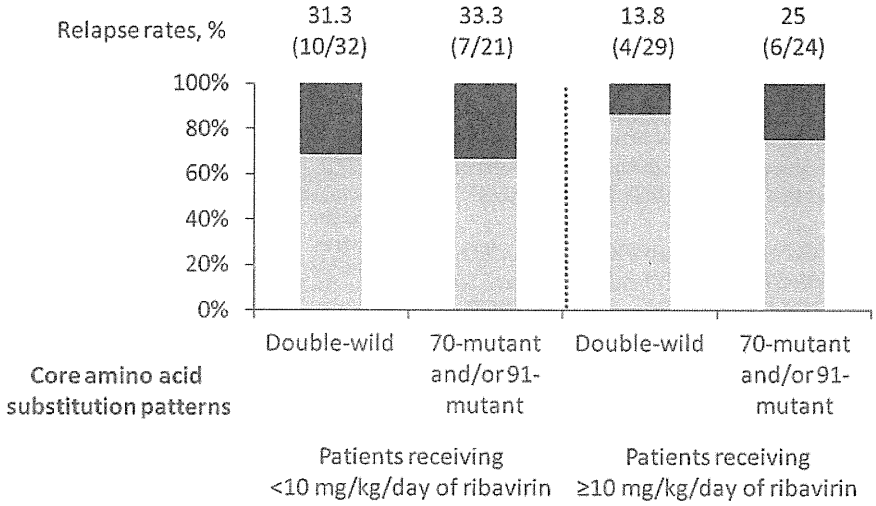

Fig. 3. Relapse rates according to core amino acid substitution patterns in patients receiving <10 mg/kg/day and receiving ≥10 mg/kg/day of ribavirin. Relapse rates are shown as percentages and the number of patients with relapse in relation to the total number of patients examined is shown at the top of each column. Gray bar, sustained virological response; black bar, relapse.

terns were similarly found in age, platelet count, and γ-GTP level. Accordingly, this study cohort had no specific bias and seems to reflect the natural background of the patients according to the HCV variance. In this study, the impact of HCV core aa substitutions on the virological response were evaluated by multivariate analysis, in order to resolve the bias of patient background factors among the groups classified according to the core aa substitution patterns. Recently, Abe et al. [2010] reported that the human genotype of the rs8099917 SNP at the IL28B locus was associated with lower γ-GTP level and viral wild type of core aa 70 and 91. Possibly these differences of IL28B genotype may influence the difference of patient background factors. Further studies are needed to clarify the relationship between human genetic variation and HCV core amino acid substitutions.

The HCV core protein has been reported to have an effect on a variety of cellular functions [Lai and Ware, 2000; Joo et al., 2005; Ariumi et al., 2007; Waris et al., 2007; Osna et al., 2008]. Currently, aa substitutions in the HCV core region has been thought to be related with outcome of antiviral therapy [Akuta et al., 2005; Donlin et al., 2007] and also the development of hepatocellular carcinoma [Akuta et al., 2007a; Hu et al., 2009]. Importance of core aa substitutions, especially at aa 70 and 91, comes to be recognized, and the new method to detect these substitutions easily has been proposed [Nakamoto et al., 2009]. As for the mechanism of antiviral activity on core aa substitutions, Ikeda et al. [2010] showed that core aa substitutions were not associated with intracellular antiviral response to IFN-alpha by in vitro analysis. The mechanism of antiviral activity and hepatocarcinogenesis on core aa substitutions has not been elucidated enough, so far. Further in vitro studies will be needed to clarify this.

Previous studies showed that patients with substitution of core aa 70 often had slow or no decrease in HCV RNA levels during the early phase of IFN-alpha treatment [Akuta et al., 2005, 2007b,c; Donlin et al., 2007]. Consistent with these reports, multivariate analysis in this study revealed that substitution of core aa 70 could be independently associated with insufficient viral decline during the first 12 weeks after the treatment (decline of <1 log from baseline at week 4, <2 log at week 12). This suggests that patients with substitution of core aa 70 are likely to fail to have a sustained virological response. On the other hand, dose exposure of Peg-IFN during the first 4 weeks of treatment was also independently linked to a minimal decline in HCV RNA (<1 log) at week 4 in this study. This suggests that maintaining the dose of Peg-IFN as high as possible until the disappearance of HCV RNA can help avoid treatment failure [McHutchison et al., 2002; Oze et al., 2009], especially in patients with substitution of core aa 70. On the other hand, substitution of core aa 91 was independently associated with detectable HCV RNA at week 24. This suggests that patients with substitution of core aa 91 are likely to achieve non-sustained virological response even if they had a ≥2 log decline in the HCV RNA level at week 12. The reason for the difference of the impact on virological response is not yet clear.

Multivariate logistic regression analysis also showed that the dose exposure of ribavirin during the full treatment period and having late virological response were independently associated with relapse. As for ribavirin exposure, it has been previously demonstrated that the relapse rate among patients responding to the treatment showed a decline in relation to the increase in the dose of ribavirin [Hiramatsu et al., 2009]. In this study, relapse rates were also decreased from 36% to 13.3% with increasing dose exposure of ribavirin among patients with end-of-treatment response. These results

confirm that maintaining a sufficient dose of ribavirin during the full treatment period could reduce the possibility of relapse, and that an extended duration of therapy for patients with late virological response could increase the chance of achieving sustained virological response, regardless of core aa substitution patterns [Berg et al., 2006; Pearlman et al., 2007; Ferenci et al., 2010].

In this study, the COBAS Amplicor HCV Test v2.0, with a lower limit of detection of 50 IU/ml, was used to assess the serum HCV RNA. Recently, real-time PCR-based HCV RNA assays with a higher sensitivity, COBAS TaqMan HCV assay (Chugai-Roche Diagnostics), with a lower limit of detection of 15 IU/ml, have been introduced. Sarrazin et al. [2010] compared virological response rates that were originally tested by COBAS Amplicor assay with those retested by COBAS TaqMan assay, using the same cohort. Among genotype 1 patients, complete early virological response and sustained virological response rates were similar when virological responses were defined as <50 IU/ml by Amplicor assay (77% and 87%) and <15 IU/ml by TaqMan assay (76% and 88%). Therefore, measuring HCV RNA by the Amplicor assay in this study would have little affects on the results.

In conclusion, the results have demonstrated that substitution of core aa 70 could be independently associated with an insufficient decline in HCV RNA level during first 12 weeks, and substitution of core aa 91 was independently associated with detectable HCV RNA at week 24, all of which were considered to be important negative predictors of attaining sustained virological response in patients with HCV genotype 1 treated with Peg-IFN plus ribavirin. On the other hand, only dose exposure of ribavirin and no complete early virological response was independent predictors of virological relapse among patients with end-of-treatment response, not substitution of core aa 70 or 91. The aa substitution patterns of the HCV core protein can be an important pretreatment predictor for non-response in patients with HCV genotype 1 treated with Peg-IFN plus ribavirin, but not for relapse after the completion of therapy.

REFERENCES

Abe H, Ochi H, Maekawa T, Hayes CN, Tsuge M, Miki D, Mitsui F, Hiraga N, Imamura M, Takahashi S, Ohishi W, Arihiro K, Kubo M, Nakamura Y, Chayama K. 2010. Common variation of IL28 affects gamma-GTP levels and inflammation of the liver in chronically infected hepatitis C virus patients. J Hepatol 53:439–443.

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007a. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 46:1357–1364.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007b. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: Amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 46:403–410.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007c. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 79:1686–1695.

Anonymous. 2002. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002—June 10–12, 2002. Hepatology 36:S3–S20.

Ariumi Y, Kuroki M, Abe K, Dansako H, Ikeda M, Wakita T, Kato N. 2007. DDX3 DEAD-box RNA helicase is required for hepatitis C virus RNA replication. J Virol 81:13922–13926.

Bedossa P, Poynard T. 1996. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 24:289–293.

Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Pape GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S. 2006. Extended treatment duration for hepatitis C virus type 1: Comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. Gastroenterology 130:1086–1097.

Blindenbacher A, Duong FH, Hunziker L, Stutvoet ST, Wang X, Terracciano L, Moradpour D, Blum HE, Alonzi T, Tripodi M, La Monica N, Heim MH. 2003. Expression of hepatitis c virus proteins inhibits interferon alpha signaling in the liver of transgenic mice. Gastroenterology 124:1465–1475.

Bode JG, Ludwig S, Ehrhardt C, Albrecht U, Erhardt A, Schaper F, Heinrich PC, Haussinger D. 2003. IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J 17:488–490.

Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. 2003. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 38:645–652.

de Lucas S, Bartolome J, Carreno V. 2005. Hepatitis C virus core protein down-regulates transcription of interferon-induced antiviral genes. J Infect Dis 191:93–99.

Dienstag JL, McHutchison JG. 2006. American Gastroenterological Association medical position statement on the management of hepatitis C. Gastroenterology 130:225–230.

Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE. 2007. Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. J Virol 81: 8211–8224.

Ferenci P, Fried MW, Shiffman ML, Smith CI, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Chaneac M, Reddy KR. 2005. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. J Hepatol 43:425–433.

Ferenci P, Laferl H, Scherzer TM, Maieron A, Hofer H, Stauber R, Gschwantler M, Brunner H, Wenisch C, Bischof M, Strasser M, Datz C, Vogel W, Loschenberger K, Steindl-Munda P. 2010. Peginterferon alfa-2a/ribavirin for 48 or 72 weeks in hepatitis C genotypes 1 and 4 patients with slow virologic response. Gastroenterology 138:503–512 e501.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347:975–982.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461: 399–401.

Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Jr., Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. 2004. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: A randomized study of treatment duration and ribavirin dose. Ann Intern Med 140:346–355.

Hiramatsu N, Oze T, Yakushijin T, Inoue Y, Igura T, Mochizuki K, Imanaka K, Kaneko A, Oshita M, Hagiwara H, Mita E, Nagase T,

Ito T, Inui Y, Hijioka T, Katayama K, Tamura S, Yoshihara H, Imai Y, Kato M, Yoshida Y, Tatsumi T, Ohkawa K, Kiso S, Kanto T, Kasahara A, Takehara T, Hayashi N. 2009. Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. J Viral Hepat 16:586–594.

Hu Z, Muroyama R, Kowatari N, Chang J, Omata M, Kato N. 2009. Characteristic mutations in hepatitis C virus core gene related to the occurrence of hepatocellular carcinoma. Cancer Sci 100:2465–2468.

Ikeda F, Dansako H, Nishimura G, Mori K, Kawai Y, Ariumi Y, Miyake Y, Takaki A, Nouso K, Iwasaki Y, Ikeda M, Kato N, Yamamoto K. 2010. Amino acid substitutions of hepatitis C virus core protein are not associated with intracellular antiviral response to interferon-alpha in vitro. Liver Int 30:1324–1331.

Joo M, Hahn YS, Kwon M, Sadikot RT, Blackwell TS, Christman JW. 2005. Hepatitis C virus core protein suppresses NF-kappaB activation and cyclooxygenase-2 expression by direct interaction with IkappaB kinase beta. J Virol 79:7648–7657.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 87:9524–9528.

Kobayashi M, Akuta N, Suzuki F, Hosaka T, Sezaki H, Suzuki Y, Arase Y, Ikeda K, Watahiki S, Mineta R, Iwasaki S, Miyakawa Y, Kumada H. 2010. Influence of amino-acid polymorphism in the core protein on progression of liver disease in patients infected with hepatitis C virus genotype 1b. J Med Virol 82:41–48.

Lai MM, Ware CF. 2000. Hepatitis C virus core protein: Possible roles in viral pathogenesis. Curr Top Microbiol Immunol 242:117–134.

Lin W, Kim SS, Yeung E, Kamegaya Y, Blackard JT, Kim KA, Holtzman MJ, Chung RT. 2006. Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. J Virol 80:9226–9235.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. 2002. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 123:1061–1069.

McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS. 2009. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 361:580–593.

Melen K, Fagerlund R, Nyqvist M, Keskinen P, Julkunen I. 2004. Expression of hepatitis C virus core protein inhibits interferon-induced nuclear import of STATs. J Med Virol 73:536–547.

Nakamoto S, Kanda T, Yonemitsu Y, Arai M, Fujiwara K, Fukai K, Kanai F, Imazeki F, Yokosuka O. 2009. Quantification of hepatitis C amino acid substitutions 70 and 91 in the core coding region by real-time amplification refractory mutation system reverse transcription-polymerase chain reaction. Scand J Gastroenterol 44:872–877.

Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H. 2009. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: A Japanese multi-center study. J Gastroenterol 44: 952–963.

Osna NA, White RL, Krutik VM, Wang T, Weinman SA, Donohue TM, Jr. 2008. Proteasome activation by hepatitis C core protein is reversed by ethanol-induced oxidative stress. Gastroenterology 134:2144–2152.

Oze T, Hiramatsu N, Yakushijin T, Kurokawa M, Igura T, Mochizuki K, Imanaka K, Yamada A, Oshita M, Hagiwara H, Mita E, Ito T, Inui Y, Hijioka T, Tamura S, Yoshihara H, Hayashi E, Inoue A, Imai Y, Kato M, Yoshida Y, Tatsumi T, Ohkawa K, Kiso S, Kanto T, Kasahara A, Takehara T, Hayashi N. 2009. 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. J Viral Hepat 16:578–585.

Pearlman BL, Ehleben C, Saifee S. 2007. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. Hepatology 46:1688–1694.

Romero-Gomez M, Del Mar Viloria M, Andrade RJ, Salmeron J, Diago M, Fernandez-Rodriguez CM, Corpas R, Cruz M, Grande L, Vazquez L, Munoz-De-Rueda P, Lopez-Serrano P, Gila A, Gutierrez ML, Perez C, Ruiz-Extremera A, Suarez E, Castillo J. 2005. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology 128: 636–641.

Sarrazin C, Shiffman ML, Hadziyannis SJ, Lin A, Colucci G, Ishida H, Zeuzem S. 2010. Definition of rapid virologic response with a highly sensitive real-time PCR-based HCV RNA assay in peginterferon alfa-2a plus ribavirin response-guided therapy. J Hepatol 52:832–838.

Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, Ichijo T, Yoshizawa K, Kiyosawa K, Tanaka E. 2008. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology 48:1753–1760.

Strader DB, Wright T, Thomas DL, Seeff LB. 2004. Diagnosis, management, and treatment of hepatitis C. Hepatology 39:1147–1171.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1104.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Thompson AJ, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. 2010. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in hepatitis C virus-1 patients. Gastroenterology 139:120–129 e118.

Waris G, Felmlee DJ, Negro F, Siddiqui A. 2007. Hepatitis C virus induces proteolytic cleavage of sterol regulatory element binding proteins and stimulates their phosphorylation via oxidative stress. J Virol 81:8122–8130.

診断と治療のTopics

C型肝炎

Hepatitis C among HIV-infected patients

独立行政法人国立病院機構大阪医療センター消化器科科長　三田英治
Eiji Mita

Summary

いまや，HIV感染者の死亡原因の第2位が肝疾患関連死である。HIV感染はC型慢性肝疾患の進展を加速させ，逆にHCV感染は抗HIV療法(ART)での肝障害の頻度・程度を悪化させる可能性があるなどHIV感染の病態を修飾する。HCVに対するワクチンがない現状では，まず感染防止，感染した場合は慢性化の阻止，慢性肝炎の場合は早期の治療介入などに重点を置いた対応が求められる。

Key words

- HCV
- インターフェロン治療
- リバビリン
- telaprevir

疫　学

C型肝炎ウイルス(hepatitis C virus：HCV)は全世界で約1億3,000万人が感染し，400～500万人がHIVと重複感染している[1]。また，アメリカではHIV感染者の約30%がHCV陽性と報告されている[2][3]。一方，日本では2004年の厚生労働省の研究班による全国調査でHIV感染者の19.2%がHCV抗体陽性で，このうち約80%がHCV-RNA陽性と報告されている[4]。日本では，HIV・HCV重複感染者の多くが血液製剤による感染が原因である。その他では，男性同性愛者間，異性間性交渉，注射による薬物乱用[5]などが感染経路である。

アメリカの大規模研究(n＝23,441)におけるHIV感染患者の死因調査で，AIDS(31.1%)に次いで肝疾患関連死(14.5%)が多いことが報告されている[6]。そして，HCVが重複感染している場合には死亡に至る相対危険度が6.7倍高値であり，C型肝炎をコントロールすることが重要である。

HCVの急性感染

HIV感染が確認されたとき，必ずHCVおよびB型肝炎ウイルス(hepatitis B virus：HBV)感染の有無は確認されているはずである。HIV感染が診断された時点でHCV感染を認めなくても，経過中にAST/ALT上昇を認めた場合はHCVマーカーを再検索するが，HCV抗体だけではなく，必ずHCV-RNAを測定すべきである。AST/ALTが異常値をとった時点でもHCV抗体が陽性化しないケースがあるため，必ずHCV-RNAまで

調べなければならない。これは，HIV感染の有無にかかわらず実施すべき手順である[7]。また，AST/ALTが正常範囲でも感染のリスク行動が継続されている場合，毎年1回HCV抗体を測定することもHCV感染を早期に把握するうえで有用といえる。

HCVは，いったん感染すると70～90%と高率にキャリア化することが知られており，HIV感染者でも同様のことがいえる。そのため，C型急性肝炎と診断された場合，治療介入を検討することになる。日本ではペグインターフェロン(peginterferon：Peg-IFN)単独治療を選択することが多く(保険適応外)，HIV非感染者ではgenotypeにかかわらず90%近いHCV排除率が得られる。しかし，HIV感染者に対し急性期にインターフェロン(interferon：IFN)導入を図った治療成績の報告は少ない。一般的には，HCV初感染が確認されて6ヵ月経過してもHCV血症が持続する場合に，慢性化と判定してIFN治療を行うことが多い。

一方，HIV・HCV感染が同時にみつかった場合，HCV感染時期を特定する情報は問診以外になく，診断に難渋する。ただ，若年者であった場合，C型肝炎の罹病期間は短いと考えられる。HCVの初感染からの期間が短いほどIFN治療効果は高いため，若年者では積極的にIFN治療を行うことを勧める。

C型慢性肝疾患の疫学

Benhamouらは[8]，HIV感染C型慢性肝炎症例と患者背景をマッチさせたHIV非感染C型慢性肝炎症例の肝線維化の進展速度を比較し，HCV単独感染例に比べHIV重複感染例は進展が速いことを報告している。肝線維化進展速度を年率で表現すると，HIV重複感染例では0.153/年であり，計算上HCV初感染から26年で肝硬変になるのに対して，HCV単独感染例では0.106/年であり，計算上HCV初感染から38年で肝硬変になるという(図1)。

またPineda[9]らは，C型肝硬変症例が非代償期に入ってからの生存期間をHIV感染の有無で検討している。HIV感染群がHIV非感染群に比し，若い(中央値 38歳 vs. 66歳)，男性が多い(86% vs. 58%)，HBs抗原陽性者が多い(24% vs. 4%)など背景因子に違いを認めるものの，平均生存期間がHIV感染群で16ヵ月，HIV非感染群で48ヵ月と，HIV感染が肝硬変の終末期においても病状悪化の一因になっていることが示されている。

C型慢性肝炎に対する抗ウイルス療法

以上のことから，HIV感染C型慢性肝炎に対しては，早期にHCV排除を目指した治療介入が望まれる。治療は，HIV感染の有無にかかわらずガイドラインに沿ったものとなる。日本人のHCVキャリアにおけるgenotypeやウイルス量の分布は，genotype 1型(ほとんどが1b型)が約70%で，高ウイルス量(≧5.0 $\log_{10}$IU/mL)が約50%，低ウイルス量(<5.0 $\log_{10}$IU/mL)が約20%という割合である。一方，残り約30%がgenotype 2型で，高ウイルス量と低ウイルス量は半数ずつという内訳である。つまり，genotype 1型・高ウイルス量症例が日本人のHCVキャリアの約半

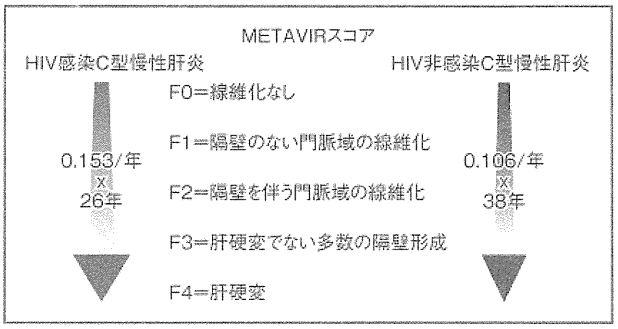

図1．HIV感染の有無とC型慢性肝炎の肝線維化進展速度

数となるが，この集団は現在の標準治療であるPeg-IFN・リバビリン併用療法をもってしても，ウイルス排除率は45～50%に留まる。他方，genotype 1型・低ウイルス量症例およびgenotype 2型症例では，ガイドラインで推奨される治療(表1)を行うことで90%近いウイルス排除率が期待できる。HIVを重複感染しているとウイルス排除率が若干抑えられるが，やはり「genotype 1型かつ高ウイルス量」症例以外であれば，病状の許すかぎり(合併症の有無，本人のモチベーションなどを考慮して)積極的にIFN治療を行うべきと考える。しかし，genotype 1型・高ウイルス量症例に対するPeg-IFN・リバビリン併用療法のウイルス排除率は低値であり[10]-[13](表2)，今後プロテアーゼ阻害薬との3剤併用療法が認可された場合は第一選択となるだろう(表1)。

次に，診療ガイドラインの要点を記載する。まず，初回治療で低ウイルス量症例なら，genotypeにかかわらずIFN単独治療もしくはPeg-IFN単独治療を行う。一方，高ウイルス量症例ならPeg-IFN・リバビリン併用療法を行い，genotype 1型なら48週治療，genotype 2型なら24週治療を行う。Genotype 1型の場合，HCV-RNAの陰性化が12週目以降36週目までなら治療期間を72週に延長する。ただ，現実的には24週目までに陰性化しないとウイルス排除率は低く，24週目の時点で継続治療の必要性を検討するべきである。HIV感染を合併する場合はウイルス排除率が低いため，海外から治療期間の工夫が提言されている[14](図2)。すなわち，4週目までにHCV-RNAの陰性化が得られなければ，genotype 1型なら72週，genotype 2型なら48週まで治療期間を延長するというものであ

表1．C型慢性肝炎に対する初回治療ガイドライン(2011年)

	genotype 1型	genotype 2型
高ウイルス量 (5.0 log_{10}IU/mL 300fmol/L 1 Meq/mL以上)	・Peg-IFNα-2b＋リバビリン(48～72週間) ・Peg-IFNα-2a＋リバビリン(48～72週間) ※ 精神症状が課題なら ・IFNβ＋リバビリン(48～72週間) ★telaprevir認可後は ・Peg-IFNα-2b＋リバビリン ＋telaprevir(24週間)	・Peg-IFNα-2b＋リバビリン(24週間) ※精神症状が課題なら ・IFNβ＋リバビリン(24週間)
低ウイルス量 (5.0 log_{10}IU/mL 300fmol/L 1 Meq/mL未満)	・IFN(24週間) ・Peg-IFNα-2a(24～48週間)	・IFN(8～24週間) ・Peg-IFNα-2a(24～48週間)

表2．HIV感染C型慢性肝炎に対するPeg-IFN・リバビリン併用療法の治療成績

	APRICOT[10]	ACTG A5071[11]	RIBAVIC[12]	Barcelona[13]
症例数	868	133	412	95
Peg-IFN	2a	2a	2b	2b
リバビリン	800mg	600～1,000mg	800mg	800～1,200mg
CD4値およびHIV-RNA	「≧200/mm^3」or「100～199/mm^3 でHIV-RNA＜5,000copies/mL」	＞100/mm^3 かつHIV-RNA＜10,000copies/mL	＞200/mm^3	＞250/mm^3 かつHIV-RNA＜10,000copies/mL
ALT	2度は上昇	不問	不問	正常上限の1.5倍以上
genotype 1型の割合	60%	77%	48%	55%
bridging fibrosisを認める慢性肝炎＋肝硬変の割合	12%	11%(肝硬変)	39%	29%
genotype 1型のウイルス排除率	29%	14%	17%	38%

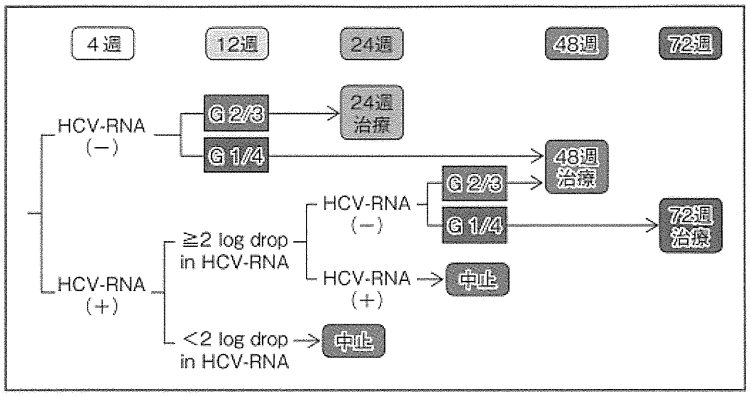

図2．HIV感染C型慢性肝炎に対するIFN治療期間の提言
G：genotype

る。また，血液製剤によってHCV感染を起こした症例も多くgenotype 3型やgenotype 4型症例を経験するが，この提言ではgenotype 3型はgenotype 2型の，genotype 4型はgenotype 1型の治療に準じている。

最近，HCV genotype 1型・高ウイルス量症例に対するPeg-IFN・リバビリン併用療法の反応を規定する因子としてインターロイキン(interleukin；IL)-28Bの一塩基多型(single nucleotide polymorphism；SNP)が報告された[15) 16)]（表3）。すなわち，IL-28BのSNP(保険適応外)がメジャーホモ接合体なら治療の反応性が良好で(厳密にいうと，経過中にリバビリンを減量することによって治療終了後に再燃するケースはあるのだが)，ヘテロ接合体もしくはマイナーホモ接合体なら治療反応性が不良である(図3)。治療反応性が不良と予測される場合は，プロテアーゼ阻害薬との3剤併用療法を行うことを検討すべきであろう。

また，抗HIV療法(antiretroviral therapy；ART)で使う薬物のなかには，リバビリンとの薬物相互作用で注意を要するものがある[17)]。リバビリンはジダノシン(ddI)の細胞内濃度を増大し，膵炎や乳酸アシドーシスを起こすが，同様のことが他の非核酸系逆転写酵素阻害薬(non-nucleoside reverse transcriptase inhibitor；NNRTI)でも観察される。また，ジドブジン(AZT)はリバビリンと併用すると高度の貧血を起こすことがあり，できれば併用を避ける。一方IFNでは，エファビレンツ(EFV)との併用で精神神経症状の増悪をきたすことがあり，できれば併用を避ける。

表3．Genotype 1型に対するPeg-IFN・リバビリン併用療法の治療効果を規定するIL-28BのSNP

SNP	rs8099917[15)]	rs12979860[16)]
メジャーホモ接合体	TT	CC
ヘテロ接合体	TG	TC
マイナーホモ接合体	GG	TT

日本人の頻度としては，メジャーホモ接合体が約4分の3，残りが4分の1の割合である。
rs8099917は真ん中に9が3つ並ぶため，トリプルナインと報告者は名付けている。一方，rs12979860はDuke大学からの報告なので，デュークスニップと学会などで呼ばれることがある。塩基配列TTは，rs8099917ではメジャーホモ接合体，rs12979860ではマイナーホモ接合体と，正反対の意味になるので注意したい。両者は連鎖不均衡を示し，同じことを示していると考えて差し支えない。

しかし，ART薬剤の進化は著しく，現在これらの薬剤は決して必須ではないため対応は可能である。今後もHCVに対する抗ウイルス薬の開発ラッシュが予定されているが，必ずART薬剤との薬理相互作用は検討されているので，情報は提供されると思われる。

C型肝硬変・肝癌に対する治療

C型肝硬変に関しては，包括的治療ガイドラインが示されている。HIV感染者に対して特別なものはなく，このガイドラインを意識した診療に努める。①治癒目的のIFN治療を行うか，②発癌予防および肝癌再発予防でIFN治療を行うか，③IFN治療を行わず(もしく

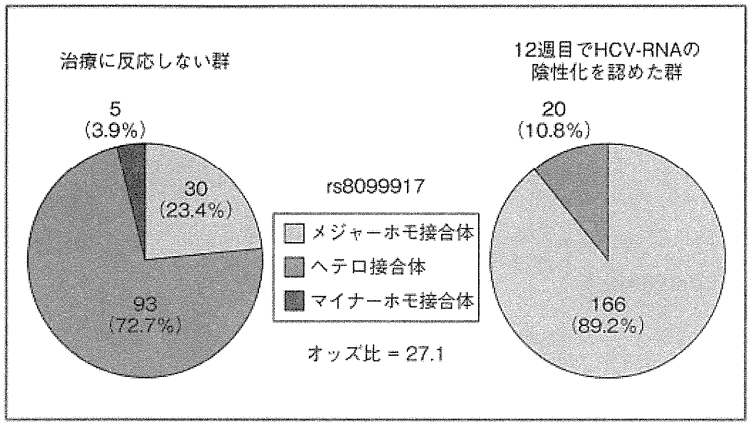

図3．IL-28BのSNP別にみたgenotype 1型に対するPeg-IFN・リバビリン併用療法の治療反応性[15]

は行えず），肝庇護療法を行うかを総合的に判断する。従来，C型肝硬変に対するIFN治療ではリバビリンを併用できなかったが，最近，治癒目的でリバビリンとの併用が保険認可された。C型肝炎のガイドラインは毎年更新されるので，日本肝臓学会のホームページなどを注視していただきたい。IFN治療ができない場合でも根気よく肝庇護療法を行い，肝病変の進展を遅らせることを目指したい。

肝癌も治療のガイドラインが示されている。早期発見には，綿密なサーベイランスが重要で，その必要性を患者に理解してもらうことが基本である。

最後に

HCV・HIV重複感染者の多くが血液製剤を介した感染で，感染期間が長くなるに伴い肝病変が進行している。適切な治療を早急に実施する必要性を感じる。ただ，現在のIFN治療は身体にかかる負担が大きい。経口の抗ウイルス薬でHCV感染を克服できる時代が早く到来することを期待したい。

文　献

1）Operskalski EA, Kovacs A：HIV/HCV co-infection；pathogenesis, clinical complications, treatment, and new therapeutic technologies. Curr HIV/AIDS Rep **8**：12-22, 2011

2）Staples CT Jr, Rimland D, Dudas D：Hepatitis C in the HIV (human immunodeficiency virus) Atlanta V.A. (Veterans Affairs Medical Center) Cohort Study (HAVACS)；the effect of coinfection on survival. Clin Infect Dis **29**：150-154, 1999

3）Anderson KB, Guest JL, Rimland D：Hepatitis C virus coinfection increases mortality in HIV-infected patients in the highly active antiretroviral therapy era；data from the HIV Atlanta VA Cohort Study. Clin Infect Dis **39**：1507-1513, 2004

4）厚生労働省科学研究費補助金エイズ対策研究事業「HIV感染症に合併する肝疾患に関する研究」班（班長 小池和彦）：平成16年度総括・分担研究報告書．2005

5）和田　清，小堀栄子：薬物依存とHIV/HCV感染―現状と対策―．日エイズ会誌 **13**：1-7，2011

6）Weber R, Sabin CA, Friis-Møller N, et al：Liver-related deaths in persons infected with the human immunodeficiency virus；the D:A:D study. Arch Intern Med **166**：1632-1641, 2006

7）藤田　実，伊藤麻里，三田英治：急性肝炎の鑑別診断と治療．必ず役立つ！肝炎診療バイブル，三田英治，加藤道夫 編著．大阪，メディカ出版，290-296，2009

8）Benhamou Y, Bochet M, Di Martino V, et al：Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. The Multivirc Group. Hepatology **30**：1054-1058, 1999

9）Pineda JA, Romero-Gómez M, Díaz-García F, et al：HIV coinfection shortens the survival of patients with hepatitis C virus-related decompensated cirrhosis. Hepatology **41**：779-789, 2005

10）Torriani FJ, Rodriguez-Torres M, Rockstroh JK, et al：Peginterferon alfa-2a plus ribavirin for chronic

hepatitis C virus infection in HIV-infected patients. N Engl J Med **351**：438-450, 2004
11) Chung RT, Andersen J, Volberding P, et al：Peginterferon alfa-2a plus ribavirin versus interferon alfa-2a plus ribavirin for chronic hepatitis C in HIV-coinfected persons. N Engl J Med **351**：451-459, 2004
12) Carrat F, Bani-Sadr F, Pol S, et al：Pegylated interferon alfa-2b vs standard interferon alfa-2b, plus ribavirin, for chronic hepatitis C in HIV-infected patients；a randomized controlled trial. JAMA **292**：2839-2848, 2004
13) Laguno M, Murillas J, Blanco JL, et al：Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for treatment of HIV/HCV co-infected patients. AIDS **18**：F27-F36, 2004
14) Soriano V, Puoti M, Sulkowski M, et al：Care of patients coinfected with HIV and hepatitis C virus；2007 updated recommendations from the HCV-HIV international panel. AIDS **21**：1073-1089, 2007
15) Tanaka Y, Nishida N, Sugiyama M, et al：Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet **41**：1105-1109, 2009
16) Ge D, Fellay J, Thompson AJ, et al：Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature **461**：399-401, 2009
17) 葛下典由：HIV 感染者の C 型肝炎．必ず役立つ！肝炎診療バイブル，三田英治，加藤道夫 編著．大阪，メディカ出版，161-163，2009

Hepatology Research 2011; 41: 626–634 doi: 10.1111/j.1872-034X.2011.00811.x

Original Article

Impact of ribavirin dose reduction on the efficacy of pegylated interferon plus ribavirin combination therapy for elderly patients infected with genotype 1b and high viral loads

Hiroshi Kohno,[1,4] Hirotaka Kouno,[1,4] Shiomi Aimitsu,[2,4] Yasuyuki Aisaka,[2,4] Mikiya Kitamoto,[3,4] Hiroiku Kawakami[4] and Kazuaki Chayama[4,5]

[1]*Department of Gastroenterology, National Hospital Organization Kure Medical Center and Chugoku Cancer Center, Kure,* [2]*Department of Hepatology, Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital,* [3]*Department of Gastroenterology, Hiroshima Prefectural Hospital,* [4]*Hiroshima IFN Study Group, and* [5]*Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan*

Aim: To examine the impact of ribavirin dose reduction on the efficacy of pegylated interferon (PEG IFN) plus ribavirin combination therapy for elderly patients infected with genotype 1b and high viral loads.

Methods: A total of 72 patients, over 65 years old, were recruited for this study. Patients were divided into groups receiving either 600–800 mg of ribavirin according to bodyweight (Group 1, $n = 36$) or 400 mg of ribavirin (Group 2, $n = 36$) plus 1.5 μg/kg (range: 1.3–2.0 μg/kg) of PEG IFN-α-2b for 48 weeks.

Results: Total ribavirin doses were administrated at 9.80 ± 2.39 mg/kg per day (3.29 ± 0.80 g/kg) for Group 1 and 5.87 ± 1.82 mg/kg per day (1.97 ± 0.61 g/kg) for Group 2 ($P < 0.001$). According to the total clearance (CL/F) of ribavirin, 34 of 36 patients in Group 1 received over-doses of ribavirin. In contrast, numbers of those receiving equivalent doses of ribavirin were two of 36 patients in Group 1 and 36 of 36 patients in Group 2, respectively ($P < 0.001$). End-of-treatment response (ETR) rates were observed in 23 of 36 patients (63.9%) in the standard ribavirin dose protocol and in 23 of 36 patients (63.9%) in the reduction ribavirin dose protocol (NS). Sustained virological response (SVR) rates were observed in 11 of 36 patients (30.6%) in the standard ribavirin dose protocol, and in 13 of 36 patients (36.1%) in the reduced ribavirin dose protocol (NS).

Conclusion: Reduction of ribavirin doses for elderly patients did not affect the outcome for the 48-week combination therapy.

Key words: elderly patients, pegylated interferon, ribavirin, total clearance

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is estimated to affect 300 million individuals worldwide[1] including 2 million people in Japan.[2] Chronic HCV infection often progresses into liver cirrhosis including the development of associated complications such as gastroesophageal varices, and hepatocellular carcinoma over the course of 20–50 years.[3,4] Pegylated interferon (PEG IFN) plus ribavirin combination therapy is currently the most effective treatment for HCV infection. Patients infected with HCV genotype 1 and high viral load are known as difficult-to-treat, resulting in a sustained virological response (SVR) of approximately 50%.[5,6] The beneficial effects of antiviral therapy in patients with chronic HCV infection include a reduction in the occurrence of hepatocellular carcinoma or hepatic disease-related mortality obtained via SVR. For the SVR, it is recommended that the patient is kept on more than

Correspondence: Dr Hiroshi Kohno, Department of Gastroenterology, National Hospital Organization Kure Medical Center and Chugoku Cancer Center, 1-3 Aoyama-cho, Kure 737-0023, Japan. Email: hkouno@kure-nh.go.jp

Received 17 November 2010; revision 14 April 2011; accepted 18 April 2011.

80% of the ribavirin dose, adjusted by the bodyweight (BW) during the combination therapy.[7] SVR rate decreased in a stepwise manner in accordance with the ribavirin dose reduction. Ribavirin might result in severe hematological adverse events when the renal function is impaired, because ribavirin concentrations increase, particularly in red blood cells. Generally, the renal function of elderly patients may naturally decrease with age.[8–10] Thus, it is difficult to obtain an SVR in elderly patients infected with genotype 1b and high viral loads.[11] In Japan, a high frequency of adverse events and high rates of discontinuation of combination therapies have also been observed in elderly patients.[12–15] Some studies have reported poor response to combination therapy in elderly patients, especially female elderly patients.[16–18] It is reported that accumulating combination of refractory factors can account for poor response rate.[18]

Thus, elderly patients with impaired renal function would often have adverse events due to ribavirin. In the present study, we examined the impact of ribavirin dose reduction on the efficacy of combination therapies for elderly patients infected with genotype 1b and high viral loads.

METHODS

Patients

THIS STUDY WAS conducted at three locations: the National Organization Kure Medical Center, Hiroshima Red Cross and Atomic Bomb Survivors Hospital, and Hiroshima Prefectural Hospital. A total of 72 patients, over 65 years old, were recruited for this study. All patients were infected with HCV genotype 1b and had high viral load of more than 5.0 log IU/mL as determined by the HCV COBAS TaqMan HCV test (Roche Diagnostics Tokyo, Tokyo, Japan). The linear dynamic range of this assay was 1.2–7.8 log IU/mL and undetectable samples were defined as negative. All eligible patients were required to satisfy the following criteria: (i) aged over 65 years; (ii) liver biopsy within 3 months of the start of therapy; (iii) diagnosis of chronic active hepatitis by conventional classification; (iv) positive for HCV RNA of genotype 1b in serum within 3 months in titers of more than 5.0 log IU/mL by the HCV COBAS TaqMan HCV test; (v) abnormal serum alanine aminotransferase levels for more than 6 months; (vi) leukocyte count of more than 3000/mm^3, platelets of more than 100 000/mm^3; (vii) serum bilirubin of less than 2.0 mg/dL; (viii) lack of liver cirrhosis, hepatocellular carcinoma, autoimmune hepatitis, alcoholic liver disease and any other chronic liver diseases (positive for serological markers of hepatitis B virus); (ix) lack of psychiatric illnesses, including depression, or conditions affecting the bone marrow, alimentary, cardiovascular or pulmonary systems; and (x) no immunosuppressive or antiviral therapy within 6 months prior to entry.

Treatment protocol

Patients were treated with the combination therapy of PEG IFN-α-2b plus ribavirin. Median dose was 1.5 μg/kg (range: 1.3–2.0 μg/kg) of PEG IFN-α-2b s.c. administrated once a week; oral ribavirin was administrated twice daily for a total dose of 400–800 mg.

The standard ribavirin dose protocol (Group 1) was as follows: 36 patients were treated for 48 weeks with a median dose of 1.5 μg/kg (range: 1.3–2.0 μg/kg) of PEG IFN-α-2b plus 600–800 mg ribavirin for patients whose weight was less or more than 60 kg, respectively.

The reduced ribavirin dose protocol (Group 2) was as follows: 36 patients were treated for 48 weeks with a median dose of 1.5 μg/kg (range: 1.3–2.0 μg/kg) of PEG IFN-α-2b plus 400 mg ribavirin.

All patients at the Kure Medical Center and Hiroshima Prefectural Hospital were enrolled in Group 1 and all patients at the Hiroshima Red Cross and Atomic Bomb Survivors Hospital were enrolled in Group 2.

In order to maintain consistency with current guidelines, patients who were HCV RNA positive by polymerase chain reaction and had abnormal alanine aminotransferase levels at 9 months were removed from the study and considered as non-responders.

This study was approved by the Institutional Review Boards of participating clinical sites prior to study initiation, and the study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all patients.

Total ribavirin clearance

Total clearance (CL/F) was calculated at the beginning of treatment using the method of Kamar *et al.*[16] as follows: CL/F (L/h) = 32.3 × BW × (1 − 0.0094 × age) × (1 − 0.42 × sex) / serum creatinine (sex = 0 for male, 1 for female). Serum ribavirin concentrations were determined by a validated high-performance liquid chromatography/tandem mass spectrometric assay using 13^C-ribavirin as an internal standard.[19,20]