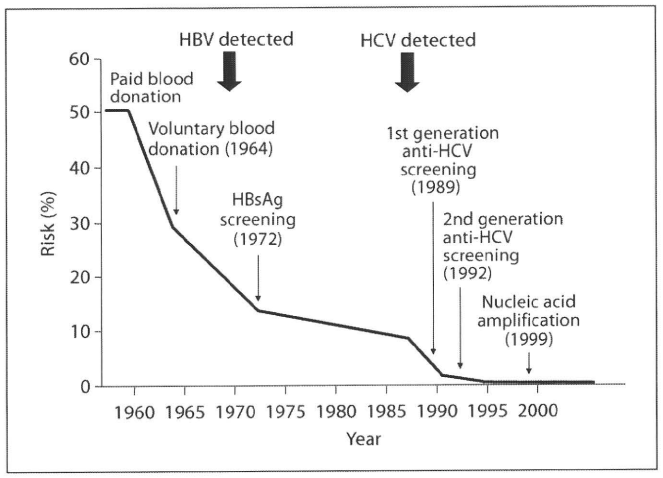

Fig. 1. Risk of post-transfusion hepatitis in Japan. Post-transfusion hepatitis occurred in more than half of transfused cases until the start of the 1960s. However, the risk has decreased significantly over the past 50 years because of the introduction of several preventive measures.

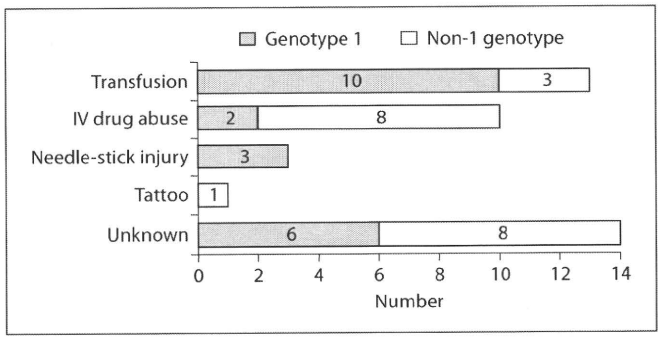

Fig. 2. Differences in transmission routes of HCV infection between genotype 1 and non-1 genotypes among patients aged less than 40 years. Intravenous drug abuse was the major route of HCV transmission in the non-1 genotypes, while blood transfusion was the major route in genotype 1.

When and How Did HCV First Spread in Japan?

Mizokami et al. [5] demonstrated based on molecular clock analysis that HCV genotype 1b, which is the most dominant genotype in Japan, started to spread in the 1930s. They also revealed that HCV genotype 1a started to spread in the United States in the 1960s, at least 30 years later than the spread of HCV genotype 1b in Japan. Furthermore, HCV infection is still increasing exponentially in the United States whereas it has been decreasing since approximately 1995 in Japan [5]. The main causes of HCV dissemination in Japan included intravenous stimulant drug (methamphetamine) abuse among the young generation during and after World War II, blood transfusion from paid blood donors, and injections using contaminated syringes and needles, particularly for the treatment of schistosomiasis japonica. Schistosomiasis japonica was previously an endemic disease in Japan before the introduction of intravenous antimony in 1921. Interestingly, HCV infection is more prevalent in south-western regions of Japan compared with north-eastern regions [4], and areas endemic for schistosomiasis japonica, such as Fukuoka and Hiroshima prefectures, are located in south-west Japan. It seems likely that the geographical distribution of schistosomiasis japonica is at least partly attributable to differences in the prevalence of HCV infection.

Resolution of HCV Transmission

Tanaka et al. [4] investigated the age-specific prevalence of HCV infection among first-time blood donors in Japan, and found that the prevalence of HCV infection is closely correlated with age. The number of HCV carriers increased with age and an exponential increase was seen in blood donors aged more than 55 years, irrespective of the area of Japan. On the other hand, the prevalence of HCV infection in blood donors younger than 30 years is quite low: 0.13% at 16–19 years and 0.21% at 20–29 years [4]. This suggests that the rate of HCV transmission has decreased significantly and that the total number of patients with HCV is likely to decrease [6–8].

Paid blood donation was commonly performed in Japan and post-transfusion hepatitis occurred in more than half of transfused cases until the beginning of the 1960s (fig. 1). After the introduction of voluntary blood donation in 1964 and HBsAg screening in 1972, the risk of post-transfusion hepatitis dramatically decreased (to around 10%). After the discovery of HCV RNA, screening tests for first- and second-generation HCV antibodies were started in 1989 and 1992, respectively. These screening tests further decreased the risk of post-transfusion hepatitis. Furthermore, nucleic acid amplification tests, introduced in 1999, almost eliminated the risk [8]. Although sporadic HCV transmission still occurs through other routes, such as intravenous drug abuse and accidental exposure in medical procedures (e.g. needlestick injury), measures to prevent post-transfusion hepatitis have significantly reduced the rate of HCV transmission.

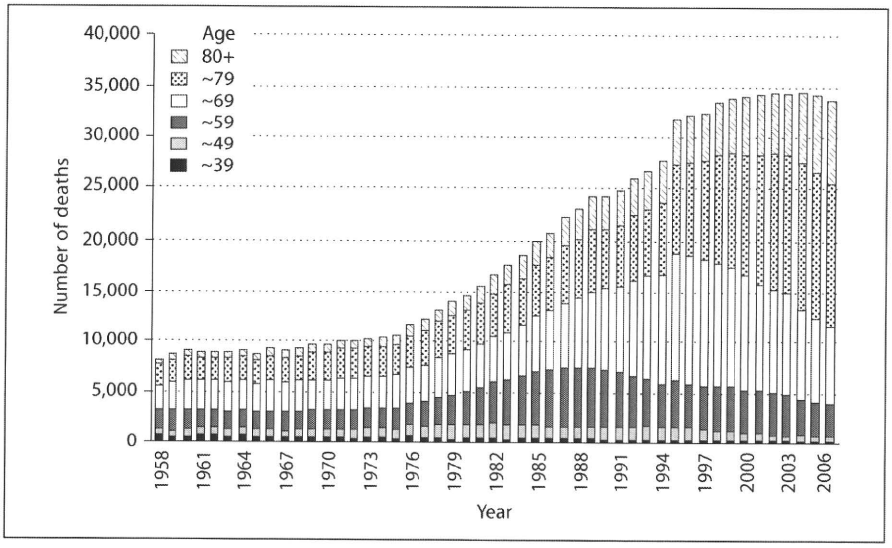

Fig. 3. Trends in mortality rates of patients with primary liver cancer. The mortality rate has steadily increased over the past 50 years, and more than 30,000 patients die of primary liver cancer every year. However, the age at death from liver cancer is also increasing.

Features of Recent HCV Transmission in Japan

As described above, HCV transmission through blood transfusion is now an extremely rare event. Instead, intravenous drug abuse has become the main cause of HCV transmission in developed areas such as Europe and the USA [9, 10]. The estimated prevalence of HCV infection among injecting drug users (IDUs) is between 30 and 80% [11–13]. The number of IDUs in Japan is steadily increasing, particularly among young people, according to the data published by the Ministry of Health, Labour and Welfare. Therefore, intravenous drug abuse has become an important route of HCV transmission among young people [8, 14]. Satoh et al. [15] investigated the HCV genotypes that are spreading among Japanese IDUs and found that non-1b genotypes of HCV, particularly genotype 2b, seemed to be most prevalent among IDUs. We also examined the HCV genotypes in 42 young (less than 40 years old) chronic hepatitis patients with HCV who were treated by peginterferon and ribavirin combination therapy at the Kinki University Hospital between 2006 and 2008. Twenty-one patients (50%) were infected with non-1 genotypes of HCV, 20 patients had genotype 2, and 1 patient had genotype 3. The proportion of non-1 genotypes in these patients is higher than that in the whole Japanese population with HCV infection, which is estimated to be about 30%. Possible transmission routes that were assessed by interviewing each patient were compared between those with genotype 1 and those with non-1 genotypes. As shown in figure 2, the major transmission routes appeared to be different between the 2 types: intravenous drug abuse was the major route of non-1 genotype HCV transmission, which is consistent with the results of previous reports [15, 16]. The sustained virological response rates in these patients, 72% for genotype 1 and 95% for non-1 genotype, are better than those in previous reports, probably due to the shorter duration of infection (associated with less advanced disease) and younger age, both of which are factors associated with favorable outcomes of IFN therapy [17, 18].

Accidental exposure to HCV during medical procedures is another important route of transmission, although the risk is believed to be very low (0.3–2.7%) [19–21]. These cases are usually monitored carefully after the accident and, even if they present with acute hepatitis, most cases can be cured by IFN therapy during the early phase of infection [22].

In summary, even though sporadic HCV infections are still occurring, particularly among young people, it seems that this population will not increase the total population of HCV infection in Japan.

Future Perspectives on HCV-Related Diseases and Mortality

In Japan, the number of HCC patients and their mortality rate remain extremely high in comparison with other developed countries [8]. Accordingly, a nationwide health screening program for viral hepatitis was launched to identify new patients with HCV or HBV infection who were unaware of their diseases. The target population of

this program included citizens aged over 40 years, and the program was carried out between 2002 and 2006. A total of 6,280,111 people (26% of the target population) were tested in this program, and 99,950 patients with HCV infection were newly detected, and some of them have already been treated with IFN therapy. Therefore, this program will provide a significant contribution to decrease the number of patients who suffer from and die of HCV-related liver diseases.

Peginterferon and ribavirin combination therapy is the current standard treatment for chronic hepatitis C infection and improves the sustained virological response rate even in patients infected with HCV genotype 1 and who have high viral load [23]. In addition, several new-generation drugs, such as protease inhibitors and polymerase inhibitors, further enhance the efficacy of IFN therapy and will be available soon [24, 25]. The improved efficacy of antiviral therapy for HCV will inevitably contribute to decrease the HCV-related mortality rate.

Although the number of patients who die of HCC has steadily increased over the past 50 years, as shown in figure 3, the incidence of HCC has recently started to decrease in Japan, mainly because of the decrease in rates of HCV-related HCC [26, 27]. In addition, the age at diagnosis of HCC has increased in parallel with the increased proportion of older patients with HCV infection [27, 28]. These trends can be explained by an estimation of the decreased number of patients with HCV infection because of the extremely low prevalence of infection in the younger generation in conjunction with improved efficacy of antiviral therapy.

Conclusion

The spread of HCV infection in Japan started in the 1930s, and widespread dissemination of the virus has occurred since then. The risk of iatrogenic HCV transmission has been almost eliminated; however, sporadic HCV transmission still occurs. The prevalence of HCV infection in the younger generation is extremely low and the total number of HCV patients is expected to decrease. Although the number of patients who die of HCC has steadily increased because of the dissemination of HCV infection in the past, it is estimated to decrease in the near future because of the decrease in rates of HCV-related HCC.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, Monden M, Kudo M: Report of the 17th nationwide follow-up survey of primary liver cancer in Japan. Hepatol Res 2007;37:676–691.

2 Yu MC, Yuan JM, Govindarajan S, Ross RK: Epidemiology of hepatocellular carcinoma. Can J Gastroenterol 2000;14:703–709.

3 El-Serag HB: Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127:27–34.

4 Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, Suzuki K, Miyakawa Y, Yoshizawa H: Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. Intervirology 2004;47:32–40.

5 Mizokami M, Tanaka Y, Miyakawa Y: Spread times of hepatitis C virus estimated by the molecular clock differ among Japan, the United States and Egypt in reflection of their distinct socioeconomic backgrounds. Intervirology 2006;49:28–36.

6 Sasaki F, Tanaka J, Moriya T, Katayama K, Hiraoka M, Ohishi K, Nagakami H, Mishiro S, Yoshizawa H: Very low incidence rates of community-acquired hepatitis C virus infection in company employees, long-term inpatients, and blood donors in Japan. J Epidemiol 1996;6:198–203.

7 Tanaka J, Mizui M, Nagakami H, Katayama K, Tabuchi A, Komiya Y, Miyakawa Y, Yoshizawa H: Incidence rates of hepatitis B and C virus infections among blood donors in Hiroshima, Japan, during 10 years from 1994 to 2004. Intervirology 2008;51:33–41.

8 Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002;62(suppl 1):8–17.

9 Sy T, Jamal MM: Epidemiology of hepatitis C virus (HCV) infection. Int J Med Sci 2006; 3:41–46.

10 Lavanchy D: The global burden of hepatitis C. Liver Int 2009;29(suppl 1):74–81.

11 Villano SA, Vlahov D, Nelson KE, Lyles CM, Cohn S, Thomas DL: Incidence and risk factors for hepatitis C among injection drug users in Baltimore, Maryland. J Clin Microbiol 1997;35:3274–3277.

12 Touzet S, Kraemer L, Colin C, Pradat P, Lanoir D, Bailly F, Coppola RC, Sauleda S, Thursz MR, Tillmann H, Alberti A, Braconier JH, Esteban JI, Hadziyannis SJ, Manns MP, Saracco G, Thomas HC, Trepo C: Epidemiology of hepatitis C virus infection in seven European Union countries: a critical analysis of the literature. Hencore group. (Hepatitis C European Network for Co-operative Research). Eur J Gastroenterol Hepatol 2000;12:667–678.

13 Mathei C, Robaeys G, van Damme P, Buntinx F, Verrando R: Prevalence of hepatitis C in drug users in Flanders: determinants and geographic differences. Epidemiol Infect 2005;133:127–136.

14 Wada K, Greberman SB, Konuma K, Hirai S: HIV and HCV infection among drug users in Japan. Addiction 1999;94:1063–1069.

15 Satoh Y, Hino K, Kato T, Mizokami M, Yamashita S, Nakamura H, Okita K: Molecular epidemiologic analysis of hepatitis C virus infection in injecting drug users with acute hepatitis C in Japan. J Gastroenterol Hepatol 2004;19:1305–1311.
16 Kato H, Maeno Y, Seko-Nakamura Y, Monma-Ohtaki J, Sugiura S, Takahashi K, Zhe LX, Matsumoto T, Kurvanov F, Mizokami M, Nagao M: Identification and phylogenetic analysis of hepatitis C virus in forensic blood samples obtained from injecting drug users. Forensic Sci Int 2007;168:27–33.
17 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: 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 2009;44:952–963.
18 Kau A, Vermehren J, Sarrazin C: Treatment predictors of a sustained virologic response in hepatitis B and C. J Hepatol 2008;49:634–651.
19 Chung H, Kudo M, Kumada T, Katsushima S, Okano A, Nakamura T, Osaki Y, Kohigashi K, Yamashita Y, Komori H, Nishiuma S: Risk of HCV transmission after needlestick injury, and the efficacy of short-duration interferon administration to prevent HCV transmission to medical personnel. J Gastroenterol 2003;38:877–879.
20 Takagi H, Uehara M, Kakizaki S, Takahashi H, Takezawa J, Kabeya K, Satoh K, Kojima A, Saito S, Matsumoto T, Hashimoto Y, Abe T, Yamada T, Konaka K, Shimoda R, Takayama H, Takehara K, Nagamine T, Mori M: Accidental transmission of HCV and treatment with interferon. J Gastroenterol Hepatol 1998;13:238–243.
21 Kiyosawa K, Sodeyama T, Tanaka E, Nakano Y, Furuta S, Nishioka K, Purcell RH, Alter HJ: Hepatitis C in hospital employees with needlestick injuries. Ann Intern Med 1991; 115:367–369.
22 Maheshwari A, Ray S, Thuluvath PJ: Acute hepatitis C. Lancet 2008;372:321–332.
23 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: 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.
24 Asselah T, Benhamou Y, Marcellin P: Protease and polymerase inhibitors for the treatment of hepatitis C. Liver Int 2009;29(suppl 1):57–67.
25 Thompson A, Patel K, Tillman H, McHutchison JG: Directly acting antivirals for the treatment of patients with hepatitis C infection: a clinical development update addressing key future challenges. J Hepatol 2009;50: 184–194.
26 Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K: Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol 2009;44(suppl 19):102–107.
27 Tanaka H, Imai Y, Hiramatsu N, Ito Y, Imanaka K, Oshita M, Hijioka T, Katayama K, Yabuuchi I, Yoshihara H, Inoue A, Kato M, Takehara T, Tamura S, Kasahara A, Hayashi N, Tsukuma H: Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. Ann Intern Med 2008; 148:820–826.
28 Taura N, Hamasaki K, Nakao K, Ichikawa T, Nishimura D, Goto T, Fukuta M, Kawashimo H, Miyaaki H, Fujimoto M, Kusumoto K, Motoyoshi Y, Shibata H, Inokuchi K, Eguchi K: Aging of patients with hepatitis C virus-associated hepatocellular carcinoma: long-term trends in Japan. Oncol Rep 2006;16: 837–843.

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Double-Filtration Plasmapheresis plus IFN for HCV-1b Patients with Non-Sustained Virological Response to Previous Combination Therapy: Early Viral Dynamics

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Key Words
Chronic hepatitis C · Double-filtration plasmapheresis · Early viral dynamics · Genotype 1b · High viral load · Interferon β · Non-sustained virological responder · Peginterferon plus ribavirin combination therapy

Abstract
Double-filtration plasmapheresis (DFPP) was approved in Japan in April 2008 for the retreatment of chronic hepatitis C patients with genotype 1b and high viral loads, whose hepatitis C virus was not eradicated by earlier IFN therapy or by pegylated IFN plus ribavirin (PEG-IFN/RBV) combination therapy. In this study, we assessed the early viral dynamics of 9 patients with non-sustained virological response to the combination therapy. The overall viral dynamics of DFPP plus IFN treatment with or without RBV for 4 weeks showed a reduction of ≥1 log in the viral load in 22% (2 of 9 patients), 55.6% (5/9), 77.8% (7/9) and 77.8% (7/9) at 24 h, 1, 2 and 4 weeks after the start of treatment. By contrast, DFPP plus consecutive intravenous IFN-β for 4 weeks reduced the viral load by ≥1 log in 33% (2/6), 50% (3/6), 83.3% (5/6) and 83.3% (5/6) at 24 h, 1, 2 and 4 weeks. The viral load declined by ≥2 log in 50% (3/6) at 4 weeks after the start of treatment. DFPP plus consecutive intravenous IFN-β for 4 weeks is a promising treatment for non-sustained virolgical response patients.

Introduction

Hepatitis C virus (HCV) infection is the major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) in industrialized countries. HCV infection is manageable, however, and its complications can be prevented by antiviral therapy [1, 2]. Currently, the most effective treatment for chronic HCV infection is based on pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy [3]. Nonetheless, sustained

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virological response (SVR) rates for those infected with the most resistant genotypes (HCV-1a and HCV-1b) still hover around 50% [3, 4].

To surmount this SVR rate with combination therapy, several trials have been undertaken, two of which are: (1) retreatment with combination therapy and (2) double-filtration plasmapheresis (DFPP). By the protocol-defined primary analysis of the former, the SVR rate has been 16% at most, even for a 72-week induction group [5].

The use of DFPP [approved in Japan in April 2008 for the retreatment of chronic hepatitis C (CHC) patients with genotype 1b and high viral loads] together with IFN administration has produced a substantial reduction in the viral load during the early stages of treatment and has effected a high SVR [6], suggesting that this treatment is a new modality for CHC patients in difficult-to-treat states. In this study, we used DFPP plus IFN to enhance the efficacy of the treatment of CHC patients whose HCV was not eradicated by earlier PEG-IFN/RBV combination therapy, and we assessed early viral dynamics associated with SVR.

Patients and Methods

Patients

Nine patients (aged 43–66 years) whose HCV had not been eradicated by earlier PEG-IFNα-2b plus RBV combination therapy carried out between 2008 and 2009 were enrolled in this study. The patients were divided into 2 groups: partial responders (PR; relapse after the end of therapy) and non-responders (NR; no disappearance of HCV RNA during therapy). All the patients were confirmed to be HCV RNA positive with high transaminase levels persisting for 6 months or longer, and with HCV RNA genotype 1b at levels exceeding 10^5 log IU/ml in blood (as determined before the start of therapy by real-time PCR). Also, the patients were negative for hepatitis B surface antigen. Patients with platelet counts of $\leq 10 \times 10^4/\mu l$, leukocyte counts of $\leq 3{,}000/\mu l$, or hemoglobin levels of ≤ 12 g/dl were excluded from the study.

Each patient gave written informed consent and agreed to receive concomitant DFPP, and the study was approved by the review board of the Kobe Asahi Hospital.

DFPP and Blood Collection

Blood collected from the peripheral vein for DFPP by a Plasmaflo™ OP-18W filter (Asahi Kasei Medical, Tokyo, Japan) was separated into plasma and cell components. The virus was then removed from the plasma by a second filter (Cascadeflo™ EC-50W; Asahi Kasei Medical) of an average pore size of 30 nm. For each session, the final volume of treated plasma was 50 ml/kg; the number of sessions was 5 over 2 weeks, and the time of DFPP, based on the reduced plasma fibrinogen levels during DFPP, was decided by the physicians and as required by the patients.

Types of IFN for 4 Weeks with DFPP

During DFPP, the patients were treated with different kinds of IFN: patient 1 with PEG-IFNα-2b plus RBV for 4 weeks; patients 2 and 3 with IFN-β 3 MU twice daily for 2 weeks and PEG-IFNα-2a plus RBV for 2 weeks; patients 4 and 9 with IFN-β 3 MU twice daily for 2 weeks and IFN-β 6 MU daily for 2 weeks; patient 5 with IFN-β 3 MU twice daily for 10 days and IFN-β 6 MU daily for 18 days, and patients 6, 7 and 8 with IFN-β 3 MU twice daily for 4 weeks. The dose of PEG-IFNα-2b was 1.5 μg/kg and 180 μg of α-2a per week. The RBV dose was 800 mg/day with α-2b and 600–800 mg/day with α-2a. After DFPP plus IFN treatment for 4 weeks, all patients were scheduled to receive PEG-IFN/RBV combination therapy (patient 1: PEG-IFNα-2b 1.5 μg/kg per week plus RBV 800 mg/day; patients 2–9: PEG-IFNα-2a 180 μg per week plus RBV 600–800 mg/day).

Amino Acid Substitutions in the Core Region (aa 30 and aa 91) and Number of IFN Sensitivity-Determining Region Mutations

We measured pre-treatment factors such as prediction of clinical outcome of therapy, amino acid sequence variation in the NS5A region (referred to as IFN sensitivity-determining regions) and in the core protein regions (aa 70 and aa 91) of HCV with a given genotype, and the viral load.

HCV RNA Measurement

The quantity of HCV RNA was measured by real-time PCR (detection limit 1.2 log IU/ml), by HCV core antigen (detection limit 20 fmol/l), and by RT-PCR (Amplicor HCV monitor v 2.0; Roche; detection limit 50 IU/ml).

Virus Removal at Second Filter Inlet and Outlet

Plasma was collected twice from the inlet and outlet of the second filter during 1 session of DFPP: once when the treated plasma volume reached half of the target quantity, and once when DFPP was completed. The change in the quantity of HCV RNA was evaluated through the plasma samples collected.

Viral Reduction and Viral Response Rate

The quantity of HCV RNA was converted to a log value at the beginning of the treatment (A) and at each of the virus measurement points (B). Δlog was then calculated: $\Delta\log = \log A - \log B = \log (A/B)$.

Evaluation of DFPP Safety

The subjective and objective adverse events of DFPP were observed, and five clinical factors were measured (platelet and lymphocyte counts, and hemoglobin, albumin and fibrinogen levels) before the first session of DFPP, before successive sessions on the second, third, fourth, fifth and sixth days, and 2 weeks after the last session.

Statistical Analysis

Statistical analysis consisted of analysis of variance for patient background factors, and the paired t test for quantities of HCV RNA at the second filter inlet during DFPP. The t test was used for viral load reductions and Fisher's exact test for viral response rates among the groups. The t test was 2-tailed, and differences of $p < 0.05$ were considered significant.

Table 1. Early viral dynamics with DFPP plus IFN treatment

Case	Age/ sex	Type of IFN for 4 weeks with DFPP	Viral dynamics after DFPP+IFN						Viral dynamics of previous treatment (PEG-IFN/RBV)				Viral mutation		
			before treatment	log drop				unit	before treatment	log drop	unit	outcome	aa 70	aa 91	ISDR
				24 h	1 wk	2 wks	4 wks			4 wks					
1	66/M	PEG-IFNα-2b/RBV 4 wks	6,510	0.5	0.6	0.6	1.1	fmol/l	452	0.7	KIU/ml	NR	wild	wild	0
2	65/F	IFN-β (3 MU 2/day) 2 wks → PEG-IFNα-2a/RBV 2 wks	7.5	0.4	1.3	2.6	1.0	log IU/ml	2,800	ND	KIU/ml	PR	wild	wild	0
3	52/F	IFN-β (3 MU 2/day) 2 wks → PEG-IFNα-2a/RBV 2 wks	5.8	0.4	1.0	1.6	+0.2	log IU/ml	6.3	0.2	log IU/ml	NR	wild	wild	1
4	47/F	IFN-β (3 MU 2/day) 2 wks → IFN-β (6 MU 1/day) 2 wks	6.8	0.6	0.3	0.4	0.4	log IU/ml	2,900	0.3	KIU/ml	NR	mutant	mutant	1
5	52/F	IFN-β (3 MU 2/day) 10 days → IFN-β (6 MU 1/day) 18 days	5.5	1.4	1.5	1.2	1.9	log IU/ml	782	0.6	fmol/l	NR	wild	mutant	1
6	61/F	IFN-β (3 MU 2/day) 4 wks	6.5	1.2	3.4	5.0	4.8	log IU/ml	8,450	2.6	fmol/l	NR	wild	wild	0
7	66/F	IFN-β (3 MU 2/day) 4 wks	5.3	0.0	0.8	1.2	1.3	log IU/ml	11,500	0.8	fmol/l	NR	mutant	wild	1
8	43/F	IFN-β (3 MU 2/day) 4 wks	3,460	0.5	0.2	1.3	2.2	fmol/l	745	0.1	fmol/l	NR	wild	mutant	1
9	43/M	IFN-β (3 MU 2/day) 2 wks → IFN-β (6 MU 1/day) 2 wks	7.2	0.6	1.4	2.5	2.9	log IU/ml	426	0.1	KIU/ml	NR	wild	wild	0

PEG-IFN/RBV: PEG-IFNα-2a (180 μg per week) plus RBV (600–800 mg/day) or PEG-IFNα-2b (1.5 μg/kg per week) plus RBV (800 mg/day). IFN-β: 3 MU twice daily or 6 MU daily. ND = Not done; aa = amino acid; ISDR = interferon sensitivity-determining region.

Results

Of the 9 patients, 1 was PR and 8 were NR. Virus mutation in the core region was as follows: wild type (7 patients) and mutant type (2 patients) at aa 70; wild type (6 patients) and mutant type (3 patients) at aa 91. IFN sensitivity-determining regions demonstrated mutation 1 (5 patients) and mutation 0 (4 patients), while mutation 2 was not seen in any patient. The overall viral dynamics of DFPP plus IFN treatment with or without RBV for 4 weeks showed a reduction in the viral load of ≥1 log in 22% (2 of 9 patients), 55.6% (5/9), 77.8% (7/9) and 77.8% (7/9) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively. The early viral dynamics after DFPP plus consecutive intravenous IFN-β treatment for 4 weeks showed a reduction in the viral load of ≥1 log in 33% (2 of 6 patients), 50% (3/6), 83.3% (5/6) and 83.3% (5/6) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively. The reduction of the viral load by ≥2 log was observed in 50% (3 of 6 patients) at 4 weeks after the start of treatment (table 1).

Discussion

New drugs to replace IFN as well as drugs that can be used in combination with IFN are being actively developed. Also, attempts are being made to find ways to physically remove HCV particles from the blood. Granulocyte apheresis, plasma exchange and hemofiltration have been applied to HCV-infected patients for the treatment of cryoglobulinemia and vasculitis, modalities which have been shown to reduce HCV RNA in the blood during treatment [6–11]. The mechanisms of the clinical results of plasmapheresis have been described, whereby HCV in the blood is related to the effects of IFN therapy that could be enhanced by removing the virus from blood [12–14]. Low-density lipoprotein-cholesterol apheresis and plasma exchange in hypercholesteremic patients with HCV infection reduces the quantity of HCV RNA in the blood of some patients [15]. Hemodialysis, hemofiltration and peritoneal dialysis in chronic dialysis patients infected with HCV significantly lower HCV RNA levels in the blood [16]. Combined granulocyte apheresis with IFN therapy for CHC [17–19] and the prerequisite for early reduction of the virus in the treatment of CHC [20, 21] are essential. Thus, the potential effectiveness of IFN therapy combined with early physical removal of the virus is of particular interest.

Asahina et al. [22] studied HCV dynamics in both serum and peripheral blood mononuclear cells in 44 patients, with HCV genotype 1b and high viral loads, randomly assigned to 4 treatment groups: (1) combination therapy with 6 MU daily of IFNα-2b plus 800 mg of RBV; (2) monotherapy with 6 MU daily of IFNα-2b; (3) monotherapy with twice-daily intravenous administration of 3 MU of IFN-β, and (4) monotherapy with daily intravenous administration of 6 MU of IFN-β. HCV RNA levels measured serially by highly sensitive real-time PCR and HCV dynamics in both serum and peripheral blood mononuclear cells have demonstrated a 'biphasic' pattern. The exponential decay slopes of the second phase have been significantly higher in the combination or the twice-daily dose regimen groups than in group 2 or 4 (0.10 ± 0.08 vs. 0.02 ± 0.09 or 0.16 ± 0.09 vs. 0.02 ± 0.04 day^{-1}; $p < 0.05$ and $p < 0.0005$, respectively) [22]. Kim et al. [23] observed that a daily dose of IFN-β 6 MU for 4 weeks effects a 2 log decrease in the HCV RNA load in 7 patients with genotype 1b and high viral loads.

In this study, early viral dynamics were assessed in the 9 patients non-SVR to the combination therapy. The overall viral dynamics of DFPP plus IFN treatment with or without RBV for 4 weeks reduced the viral load by ≥1 log in 22% (2 of 9 patients), 55.6% (5/9), 77.8% (7/9), and 77.8% (7/9) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively. DFPP plus consecutive intravenous IFN-β treatment for 4 weeks reduced the viral load by ≥1 log in 33% (2/6), 50% (3/6), 83.3% (5/6) and 83.3% (5/6) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively.

The prerequisite for early virological response (EVR; indicating negative HCV RNA at 12 weeks) has been emphasized in predicting SVR and non-SVR in CHC patients undergoing IFN treatment; those who do not reach EVR fail to respond to further therapy. Treatment discontinued in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for CHC is highly desirable [24].

To be able to predict SVR with PEG-IFN/RBV treatment, reduction of the HCV RNA viral load by week 4 is considered essential. A 2 log reduction in the HCV RNA viral load by week 4 is a prerequisite to achieving SVR with PEG-IFN/RBV treatment [25]. In our study of DFPP plus consecutive intravenous IFN-β treatment for 4 weeks, a reduction in the viral load of ≥2 log was achieved in 50% (3 of 6 patients) at 4 weeks after the start of treatment.

From the above considerations, DFPP plus consecutive intravenous IFN-β treatment for 4 weeks is a promising regimen for non-SVR patients with genotype 1b and high viral loads, previously treated with PEG-IFN/RBV therapy. Further study is needed to elucidate the SVR rate in a larger number of patients given DFPP plus IFN treatment, especially with consecutive intravenous IFN-β.

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Disclosure Statement

No conflict of interest exists.

References

1 Hoofnagle JH, Seeff LB: Peginterferon and ribavirin for chronic hepatitis C. N Engl J Med 2006;355:2444–2451.
2 Pawlotsky JM: Therapy of hepatitis C: from empiricism to eradication. Hepatology 2006; 43:S207–S220.
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 FL Jr, et al: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347:975–982.
5 Jensen DM, Freilich B, Andreone P, et al: Pegylated interferon alfa-2A (40KD) plus ribavirin (RBV) in prior non-responders to pegylated interferon alfa-2B (12KD)/RBV: final efficacy and safety outcomes of the repeat study. Hepatology 2007;46(suppl 1):291–292.
6 Fujiwara K, Kaneko S, Kakumu S, et al: Double filtration plasmapheresis and interferon therapy for chronic hepatitis C patients with genotype 1 and high viral load. Hepatol Res 2007;37:701–710.
7 Fabrizi F, Martin P, Dixit V, et al: Biological dynamics of viral load in hemodialysis patients with hepatitis C virus. Am J Kidney Dis 2000;35:122–129.
8 Manzin A, Candela M, Solforosi L, Gabrielli A, Clementi M: Dynamics of hepatitis C viremia after plasma exhange. J Hepatol 1999; 31:389–393.
9 Ramratnam B, Bonhoeffer S, Binley J, et al: Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. Lancet 1999;354: 1782–1785.
10 Schettler V, Monazahian M, Wieland E, Thomssen R, Muller GA: Effect of heparin-induced extracorporeal low-density lipoprotein precipitation (HELP) apheresis on hepatitis C plasma virus load. Ther Apher 2001;5: 384–386.

11 Schettler V, Monazahian M, Wieland E, et al: Reduction of hepatitis C virus load by H.E.L.P.-LDL apheresis. Eur J Clin Invest 2001;31:154–155.
12 Sakai A, Kaneko S, Matsushita E, Kobayashi K: Floating density of hepatitis C virus particles and response to interferon treatment. J Med Virol 1998;55:12–17.
13 Sakai A, Kaneko S, Kobayashi K: Immunoadsorption therapy for HCV infected chimpanzee. Nippon Rinsho 2001;59:1374–1378.
14 Yamashita T, Arai K, Sakai A, et al: Virological effects and safety of combined double filtration plasmapheresis (DFPP) and interferon therapy in patients with chronic hepatitis C: a preliminary study. Hepatol Res 2006;36:167–175.
15 Marson P, Boschetto R, De Silvestro G, et al: Changes in HCV viremia following LDL apheresis in a HCV positive patient with familial hypercholesterolemia. Int J Artif Organs 1999;22:640–644.
16 Ishida H, Tanabe K, Tokumoto T, et al: Hepatitis C virus decrease in patients with maintenance hemofiltration therapy. Artif Organs 2004;28:316–318.
17 Diepolder HM, Kashiwagi N, Teuber G, et al: Leucocytapheresis with Adacolumn enhances HCV-specific proliferative responses in patients infected with hepatitis C virus genotype 1. J Med Virol 2005;77:209–215.
18 Sawada K, Masaki N, Hayashi S, et al: Immunomodulatory effects of selective leucocytapheresis as a new adjunct to interferon-α2b plus ribavirin combination therapy: a prospective study in patients with high plasma HCV viraemia. J Viral Hepat 2005;12:274–282.
19 Moriyama M, Kaneko M, Matsumura H, et al: Removal of hepatitis C virus by G-1 beads in sera from patients with chronic hepatitis C. Intervirology 2005;48:84–88.
20 Hayashi N, Takehara T: Antiviral therapy for chronic hepatitis C: past, present, and future. J Gastroenterol 2006;41:17–27.
21 Ballesteros AL, Fuster D, Planas R, Clotet B, Tural C: Role of viral kinetics under HCV therapy in HIV/HCV-coinfected patients. J Antimicrob Chemother 2005;55:824–827.
22 Asahina Y, Izumi N, Uchihara M, et al: A potent antiviral effect on hepatitis C viral dynamics in serum and peripheral blood mononuclear cells during combination therapy with high-dose daily interferon alfa plus ribavirin and intravenous twice-daily treatment with interferon beta. Hepatology 2001;34:377–384.
23 Kim KI, Sasase N, Taniguchi M, et al: Interferon-β induction/interferon-α2b plus ribavirin therapy in patients with chronic hepatitis C. Int J Clin Pharm Res 2005;25:71–76.
24 Davis GL: Monitoring of viral levels during therapy of hepatitis C. Hepatology 2002;36:S145–S151.
25 Nomura H, Miyagi Y, Tanimoto H, Higashi M, Ishibashi H: 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. J Gastroenterol 2009;44:338–345.

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Outcome and Early Viral Dynamics with Viral Mutation in PEG-IFN/RBV Therapy for Chronic Hepatitis in Patients with High Viral Loads of Serum HCV RNA Genotype 1b

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Key Words

Chronic hepatitis · Early viral dynamics · IFN/RBV resistance-determining region · HCV RNA genotype 1b · High viral load · PEG-IFN/RBV combination therapy · Virological response, prediction

Abstract

We investigated whether sustained virological response (SVR) and non-SVR by chronic hepatitis C patients to pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy are distinguishable by viral factors such as the IFN/RBV resistance-determining region (IRRDR) and by on-treatment factors through new indices such as the rebound index (RI). The first RI (RI-1st; the viral load at week 1 divided by the viral load at 24 h) and the second RI (RI-2nd; the viral load at week 2 divided by the viral load at 24 h) were calculated. The subject patients were divided into 3 groups based on RI-1st and RI-2nd: an RI-A group (RI-1st ≤1.0), an RI-B group (RI-1st >1.0 and RI-2nd <0.7) and an RI-C group (RI-1st >1.0 and RI-2nd ≥0.7). The SVR rate was 71.4% (10/14) in the RI-A group, 46.2% (6/13) in the RI-B group and 20.0% (3/15) in the RI-C group (p = 0.005 between the RI-A group and the RI-C group). In IRRDR ≥6 and IRRDR ≤5 the SVR rate was 81.3% (13/16) and 23.1% (6/26) (p = 0.0002), respectively. By combining RI and IRRDR as a predicting factor, the SVR rate was 87.5% (7/8) in the RI-A group (≥6 mutations in the IRRDR) and 7.7% (1/13) in the RI-C group (≤5 IRRDR mutations) (p = 0.0003).

Introduction

Recently, global consensus has obtained that a combination of IFN or pegylated IFN plus ribavirin (PEG-IFN/RBV) is the treatment of choice for chronic hepatitis C (CHC). Notwithstanding this treatment regimen, sustained virological response (SVR) rates of those infected with the most resistant genotypes [hepatitis C virus (HCV)-1a and -1b] still hover at ~50% [1, 2]. It is therefore worthwhile to identify the predictive factors that allow the selection of patients who would achieve eradication

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of HCV RNA either before or during therapy, especially since IFN/RBV combination therapy is costly and has several side effects [3].

Predictors of the effectiveness of IFN-based therapy can be classified into pretreatment and on-treatment factors. Pretreatment factors comprise: (1) host factors such as age, gender, obesity, alcohol consumption, hepatic iron overload, fibrosis, immune responses and co-infection with other viruses, and (2) viral factors that mainly include viral genotypes and loads, particular amino acid sequence variations in the NS5A region [4, 5] and in the core protein region of HCV [6] within a given genotype. Moreover, the mean number of mutations in variable region 3 (V3) plus its upstream flanking region of NS5A [amino acid 2334–2379, referred to as IFN/RBV resistance-determining region (IRRDR)] is significantly higher in HCV isolates obtained from patients who later achieve SVR by PEG-IFN/RBV than in those from non-SVR patients. On-treatment factors are mainly related to viral kinetics within the first few weeks of treatment [7].

In the current study, with the aim of investigating whether SVR and non-SVR can be distinguished by viral factors such as IRRDR and by on-treatment factors through new indices such as the rebound index (RI), we calculated the first RI (RI-1st; the viral load at week 1 divided by the viral load at 24 h) and the second RI (RI-2nd; the viral load at week 2 divided by the viral load at 24 h), as proposed by Nomura et al. [8].

Patients and Methods

The 42 patients included in this study, who all demonstrated high viral loads (>100 KIU/ml) of serum HCV RNA of genotype 1b, had been diagnosed with CHC on the basis of abnormal serum alanine aminotransferase persisting for at least 6 months, and of positive HCV RNA assessed by RT-PCR. None of the patients was positive for hepatitis B surface antigen or other liver diseases (autoimmune hepatitis, alcoholic liver disease). All the patients received a regimen of PEG-IFNα-2b (peginterferon alpha-2b; PegIntron; Schering-Plough, Kenilworth, N.J., USA) (1.5 μg/kg/week, subcutaneously) in combination with RBV (ribavirin; Rebetol; Schering-Plough) 600–1,000 mg/day for 48 weeks. RBV was administered at a dose of 600 mg/day (3 capsules) to patients weighing <60 kg, 800 mg/day (4 capsules) to those weighing <80 kg and 1,000 mg/day (5 capsules) to those weighing ≥80 kg.

The efficacy of the combination therapy was evaluated by HCV RNA negativity determined by qualitative RT-PCR analysis at the end of therapy (end of therapy response) and 6 months after the completion of therapy (SVR). The amount of HCV RNA was also measured quantitatively by RT-PCR (Amplicor HCV monitor v. 2.0; Roche) before therapy. The lower detection limit of the assay was 5 KIU/ml. Samples collected during and after therapy were also determined by qualitative RT-PCR (Amplicor; Roche), which has a higher sensitivity than quantitative analysis, and the results were labeled as positive or negative. The lower limit of the assay was 50 IU/ml.

SVR was defined as undetectable serum HCV RNA at 24 weeks after the cessation of treatment, and non-SVR as detectable HCV RNA at 24 weeks after the discontinuation of treatment. Informed consent was obtained from all patients enrolled in the study after thoroughly explaining the aims, risks and benefits of the therapy.

The amount of HCV core antigen was assessed by the IRM assay (Ortho Clinical Diagnostics, Tokyo, Japan), which provides a good correlation between the amount of HCV core antigen and the amount of HCV RNA, as shown in our previous study [9]. The HCV core antigen was measured on days 0, 1 (24 h), 7 (1 week) and 14 (2 weeks) according to the detection limit of 20 fmol/l established by the manufacturer.

RI-1st was defined as the coefficient derived by dividing the viral load of HCV core antigen at week 1 by that at 24 h, and RI-2nd was defined as the coefficient derived by dividing the viral load at week 2 by that at 24 h [8].

The patients were divided into 3 groups based on RI-1st and RI-2nd: group A (RI-1st ≤1.0), group B (RI-1st >1.0 and RI-2nd <0.7) and group C (RI-1st >1.0 and RI-2nd ≥0.7).

NS5A sequence analysis (IRRDR) was performed as described [4]. Briefly, the sequences of the amplified fragments were determined by direct sequencing without subcloning with the use of a Big Dye Deoxy Terminator cycle sequencing kit and an ABI 337 DNA sequencer (Applied Biosystems, Japan). The aa sequences were deduced and aligned with Genetyx Win software v. 7.0 (Genetyx Corp., Tokyo, Japan). Numbering of aa throughout the manuscript is according to the polyprotein of HCV genotype 1b prototype HCV-J.

Statistical Analysis

Differences between the groups were assessed by the χ^2 test, Fisher's exact test or Student's t test, the Mann-Whitney test and the Kruskal-Wallis test. $p < 0.05$ was considered statistically significant.

Results

Of the 42 patients treated with combination therapy, 19 (45.2%) achieved SVR and 23 (54.8%) were still HCV RNA positive (non-SVR) 6 months after therapy. No significant differences were observed in patient characteristics between SVR and non-SVR, except in platelet counts and the degree of fibrosis (table 1), or among the RI-A, -B and -C groups (table 2).

The SVR rate was 71.4% (10/14), 46.2% (6/13) and 20.0% (3/15) in the RI-A, -B and -C groups, respectively, with a significant difference between the RI-A and -C groups ($p = 0.005$), but not significant between the RI-A and -B groups and the RI-B and -C groups (fig. 1). In the 14 patients of the RI-A group, HCV RNA turned negative

Table 1. Host-dependent, virus-related profile by response (SVR and non-SVR)

	SVR	Non-SVR	p value
Gender (M/F), n	11/8	13/10	NS
Age, years	56.7 ± 8.8	59.3 ± 10.5	NS
HCV RNA level, KIU/ml	1,685 ± 1,477	1,660 ± 1,363	NS
HCV core antigen, fmol/l	7,044 ± 6,763	9,343 ± 12,563	NS
Body weight, kg	59.9 ± 11.5	59.8 ± 13.6	NS
Treatment history (retreatment/naïve)	6/13	13/10	NS
Platelet count ($\times 10^4/mm^3$)	18.7 ± 4.4	14.8 ± 5.4	0.02
F0, 1/F2, 3	12/2	5/10	0.004

Table 2. Host-dependent, virus-related profile by response (RI-A, -B and -C groups)

	RI-A	RI-B	RI-C	p value
Gender (M/F), n	7/7	9/4	8/7	NS
Age, years	60.0 ± 5.9	58.5 ± 9.4	56.1 ± 12.8	NS
HCV RNA level, KIU/ml	1,401 ± 1,014	2,053 ± 1,286	1,593 ± 1,772	NS
HCV core antigen, fmol/l	6,084 ± 5,106	7,674 ± 5,038	11,000 ± 15,837	NS
Body weight, kg	62.1 ± 16.6	59.5 ± 10.4	58.2 ± 10.1	NS
Treatment history (retreatment/naïve)	3/11	7/6	9/6	NS
Platelet count ($\times 10^4/mm^3$)	15.3 ± 3.5	18.3 ± 5.9	16.3 ± 6.0	NS
F0, 1/F2, 3	7/3	5/4	5/5	NS

Table 3. SVR rate between IRRDR ≤5 and IRRDR ≤6 in RI-A, -B and -C groups

	RI-A		RI-B		RI-C	
	IRRDR ≤5	IRRDR ≥6	IRRDR ≤5	IRRDR ≥6	IRRDR ≤5	IRRDR ≥6
SVR	3	7	2	4	1	2
Non-SVR	3	1	5	2	12	0
SVR rate, %	50.0	87.5	28.6	66.7	7.7	100
p value	NS		NS		0.0024	
	0.0003					

by week 4 in 3 patients, week 8 in 5 patients, week 12 in 5 patients and was positive in 1 patient throughout the treatment. In the 13 patients of the RI-B group, HCV RNA was negative by week 4 in 1 patient, week 8 in 2 patients, week 12 in 4 patients, at and after week 16 in 5 patients and remained positive throughout the treatment in 1 patient. In the 15 patients of the RI-C group, HCV RNA was negative by week 12 in 1 patient, on and after week 16 in 6 patients and remained positive throughout the treatment in 8 patients (fig. 2).

The SVR rate was 81.3% (13/16) in the group with ≥6 mutations in IRRDR, and 23.1% (6/26) in those with ≤5 (fig. 3), with a significant difference between the 2 groups ($p = 0.0002$).

By combining RI and IRRDR, the SVR rate was 87.5% (7/8) in the RI-A group (IRRDR ≥6) and 7.7% (1/13) in the RI-C group (IRRDR ≤5) (table 3), with a significant difference between the 2 groups ($p = 0.0003$).

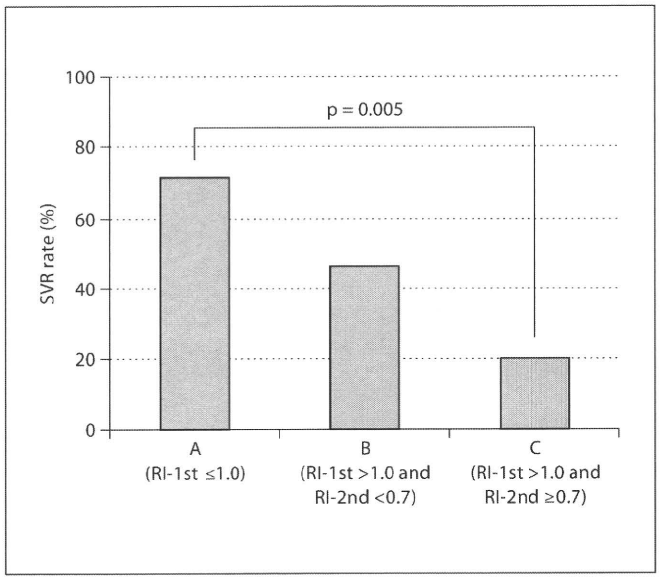

Fig. 1. SVR rate in RI-A, -B and -C groups. The overall SVR rate was 71.4, 46.2 and 20.0%, respectively. Significant difference in SVR rate is indicated.

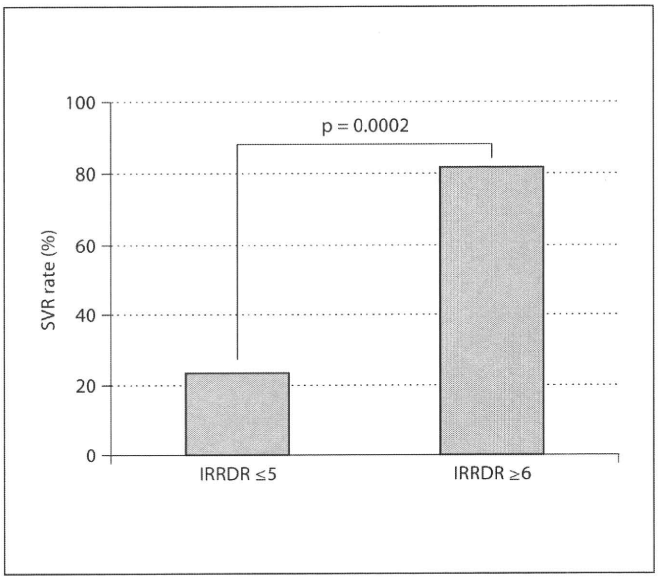

Fig. 3. SVR rate and IRRDR number. The SVR rate was 23.1% in IRRDR ≤5 and 81.3% in IRRDR ≥6, which was significantly different.

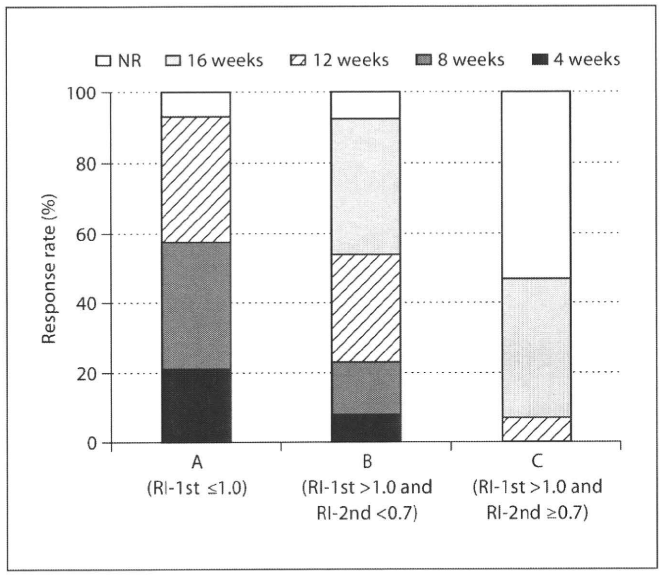

Fig. 2. Relation between response time and virus dynamics. In the 14 patients of the RI-A group, HCV RNA turned negative by week 4 in 3 patients, week 8 in 5 patients, week 12 in 5 patients and remained positive throughout the treatment in 1 patient. In the 13 patients of the RI-B group, HCV RNA was negative by week 4 in 1 patient, week 8 in 2 patients, week 12 in 4 patients, at and after week 16 in 5 patients and remained positive throughout the treatment in 1 patient. In the 15 patients of the RI-C group, HCV RNA was negative by week 12 in 1 patient, at and after week 16 in 6 patients and remained positive throughout the treatment in 8 patients.

Discussion

The importance of early virological response (EVR; signifying HCV RNA negative at 12 weeks) has been emphasized in predicting SVR and non-SVR in CHC patients undergoing IFN treatment; those not reaching EVR do not respond to further therapy. Discontinuation of treatment in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for CHC is worth investigating [10].

Treatment with IFN induces a decline in HCV RNA levels that can be mathematically measured in 2 phases. The decline in the first phase, usually measured at 24 or 48 h, probably reflects direct inhibition of intracellular production and release of HCV [11], with IFN efficacy ranging from about 70% (approx. 0.7 log units) for standard IFN (given 3 times a week) to more than 90% (1 log unit) for high daily doses of standard IFN or PEG-IFN (given once a week) [12, 13]. The decline in the second phase, beginning after 24–48 h, is slower and more variable than that in the first phase, and is thought to reflect continued inhibition of replication and the gradual elimination of virus-infected cells [11]. The decay in the first phase has little correlation with the IFN dose, but is more rapid with PEG-IFN than with standard IFN preparations [10].

Lowering HCV RNA during the first phase is essential for efficient elimination of HCV during the second phase. Decreases in HCV RNA titers within the first 24–48 h after the start of IFN would, therefore, be a dependable estimate of antiviral efficacy [12, 13].

Early viral kinetics, determined up to week 2, are believed to express the therapeutic effect of PEG-IFN. The concentration of PEG-IFNα-2b in serum peaks after 24 h, then declines gradually [14, 15]. The viral load is thus reduced by 24 h but increases in week 1 [16, 17]; with a large dose of PEG-IFN at each administration, it decreases markedly at 24 h but then increases in week 1 regardless of the dose. In the responder group, however, the viral load continues to decline each week thereafter [17].

In this study, we used new indices proposed by Nomura et al. [8]: RI-1st and RI-2nd calculated from early viral kinetics. RI-1st was defined as the coefficient derived by dividing the viral load of HCV core antigen at week 1 by that at 24 h, and the RI-2nd was defined as the coefficient derived by dividing the viral load at week 2 by that at 24 h. In the SVR group, a number of patients demonstrated no increase in the viral load at week 1. Patients with a high RI-2nd were regarded as poor responders or non-responders to PEG-IFN. The RI-2nd of those other than non-responders was below 0.7; therefore, 0.7 was adopted as the reference value for RI-2nd, and the patients were divided into 3 groups based on RI-1st and RI-2nd: the RI-A group (RI-1st $\leq$1.0), the RI-B group (RI-1st >1.0 and RI-2nd <0.7) and the RI-C group (RI-1st >1.0 and RI-2nd $\geq$0.7). The SVR rate of the RI-A, RI-B and RI-C groups was 71.4% (10/14), 46.2% (6/13) and 20% (2/10), respectively ($p = 0.005$ between the RI-A group and the RI-C group). RIs are also associated with the early clearance of HCV RNA that is related to SVR.

In the RI-A group 21.4% (3/14), 35.7% (5/14) and 35.7% (5/14) became HCV RNA negative by weeks 4, 8 and 12, respectively. In the RI-B group 7.7% (1/13), 15.4% (2/13), 30.8% (4/13) and 38.5% (5/13) became HCV RNA negative by weeks 4, 8, 12, and at and after week 16, respectively. In the RI-C group 6.7% (1/15) and 40.0% (6/15) became HCV RNA negative by week 12, and at and after week 16, respectively. It is believed that the simplified RI-1st and RI-2nd are evidential indices for determining the therapeutic efficacy of PEG-IFN/RBV treatment.

We have previously reported that the high degree of sequence variation in IRRDR (IRRDR $\geq$6) significantly correlates with SVR, whereas the low degree of sequence variation in this region (IRRDR $\leq$5) correlates with non-SVR [4]. A significant correlation between the rapid reduction of HCV core antigen titers and the degree of sequence variation in IRRDR has been observed. This, in particular, suggests a possible influence of IRRDR $\geq$6 on HCV replication kinetics during IFN-based therapy, especially that the direct effect of IFN begins a few hours after the first dose.

In this study, the SVR rate was 81.2% (13/16) with IRRDR $\geq$6 and 23.1% (6/26) with IRRDR $\leq$5 ($p = 0.0002$), strongly suggesting that IRRDR $\geq$6 would be a useful marker for the prediction of SVR.

By combining RI and IRRDR as a predicting factor, the SVR rate was 87.5% (7/8) in the RI-A group (RI-1st $\leq$1.0) with IRRDR $\geq$6, signifying that about 90% of these patients turned SVR and were, thus, believed to be very good responders. An SVR rate of 7.7% (1/13) was obtained in the RI-C group with IRRDR $\leq$5 ($p = 0.0003$).

In conclusion, we propose that IRRDR combined with RIs is the most sensitive predictive factor for SVR and non-SVR. With the aid of RIs and IRRDR, a more effective PEG-IFN/RBV treatment could be within reach. A more detailed investigation with a larger number of subjects is needed to confirm the current results in patients given PEG-IFN/RBV combination therapy.

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Disclosure Statement

No conflict of interest exists.

References

1 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.

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

3 Nakamura H: Early prediction of sustained viral responder and non-responder during interferon and ribavirin combination therapy in chronic hepatitis C. Hepatol Res 2005; 33:269–271.

4 El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H: Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. Hepatology 2008;48:38–47.
5 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996; 334:77–81.
6 Akuta N, Suzuki S, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Miyakawa Y, Kumada H: Prediction of response to pegylated interferon and ribavirin in hepatitis C by polymorphism in the viral core protein and very early dynamics of viremia. Intervirology 2007;50:361–368.
7 Ferenci P: Predictors of response to therapy for chronic hepatitis C. Semin Liver Dis. 2004;24:S25–S31.
8 Nomura H, Miyagi Y, Tanimoto H, Higashi M, Ishibashi H: 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. J Gastroenterol 2009;44:338–345.
9 Sasase N, Kim SR, Kim KI, Taniguchi M, Imoto S, Hotta H, Shouji I, El-Shamy A, Kawada N, Kudo M, Hayashi Y: Usefulness of a new immunoradiometric assay of HCV core antigen to predict virological response during PEG-IFN/RBV combination therapy for chronic hepatitis with high viral load of serum HCV RNA genotype 1b. Intervirology 2008;51:S70–S75.
10 Davis GL: Monitoring of viral levels during therapy of hepatitis C. Hepatology 2002;36: S145–S151.
11 Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS: Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-α therapy. Science 1998;282:103–107.
12 Lam NP, Neumann AU, Gretch DR, Wiley TE, Perelson AS, Layden TJ: Dose-dependent acute clearance of hepatitis C genotype 1 virus with interferon alfa. Hepatology 1997;26:226–231.
13 Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, Roth WK: Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alfa-2a. Gastroenterology 2001;120: 1438–1447.
14 Silva M, Poo J, Wagner F, Jackson M, Cutler D, Grace M, et al: A randomized trial to compare the pharmacokinetic, pharmacodynamic, and antiviral effects of peginterferon alfa-2b and peginterferon alfa-2a in patients with chronic hepatitis C (COMPARE). J Hepatol 2006;45:204–213.
15 Asahina Y, Izumi N, Umeda N, Hosokawa T, Ueda K, Doi F, 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.
16 Izumi N, Asahina Y, Kurosaki M, Uchihara M, Nishimura Y, Inoue K, 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.
17 Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M, 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.

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Prolonged PEG-IFN and RBV Is Effective in Patients with HCV Genotype 1 and High Viral Load Who Achieved Virological Response Later than 24 Weeks

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Key Words
Genotype 1 · Hepatitis C virus · High viral load · Late virological responder · Peginterferon · Ribavirin · Sustained virological response

Abstract
Objective: The extension of treatment duration has been proposed in late virological responders with hepatitis C virus (HCV) genotype 1 and high viral load. However, the effectiveness of extended treatment in patients whose serum HCV RNA become undetectable later than 24 weeks of treatment (ultra-late virological responder; ULVR) has not yet been determined. ***Methods:*** A total of 130 patients infected with HCV genotype 1 and who had high viral load were treated with pegylated interferon (PEG-IFN) and ribavirin (RBV) combination therapy. We retrospectively analyzed 10 ULVR who received extended combination treatment beyond 48 weeks. ***Results:*** The duration of the combination treatment for ULVR ranged between 59 and 119 weeks, and the mean duration was 80 weeks. Although the majority of ULVR were older female patients (≥60 years) with factors related to poor therapeutic response, 8 patients (80%) achieved sustained virological response (SVR). The SVR rate correlated well with the duration of the treatment. Five ULVR achieved SVR when treatment was continued until serum HCV RNA remained undetectable for longer than 48 weeks. ***Conclusion:*** The extended duration of PEG-IFN and RBV combination treatment is a possible strategy to improve treatment response in HCV genotype 1 infection, even for ULVR.

Introduction

Peginterferon (PEG-IFN) and ribavirin (RBV) combination treatment is the standard treatment for chronic hepatitis C infection that results in improved sustained virological response (SVR), even in patients with hepatitis C virus (HCV) genotype 1 and high viral load [1, 2]. Recently, an extension of the treatment duration to 72 weeks has been proposed for late virological responders who have a 2-log decrement in HCV RNA from baseline at 12 weeks of the treatment and undetectable serum HCV RNA after 13–24 weeks of the treatment [3, 4]. However, the benefit of the extended combination treat-

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ment in patients whose serum HCV RNA become undetectable later than 24 weeks of the treatment (ultra-late virological responder; ULVR) has not yet been determined.

In the present study, we addressed the effectiveness and feasible duration of the extended PEG-IFN and RBV combination treatment in ULVR infected with HCV genotype 1 and who had a high viral load.

Patients and Methods

A total of 130 patients infected with HCV genotype 1 and who had a high viral load were treated with PEG-IFNα-2b (Peg-Intron; Schering-Plough, Kenilworth, N.J., USA) and RBV (Rebetol; Schering-Plough) combination treatment between January 2005 and August 2008 at the Kinki University Hospital. PEG-IFNα-2b was administered at 1.5 μg/kg subcutaneously each week and RBV was administered orally at a dose of 600 mg to patients weighing <60 kg, 800 mg to those weighing 60–80 kg, and 1,000 mg to those weighing ≥80 kg daily. The dose reduction and discontinuation of the combination treatment was determined according to standard protocols. The total duration of the treatment was determined by the attending physicians or by patient request. The serum HCV RNA was measured before commencement of treatment and every month during the treatment by quantitative Amplicor HCV monitor assay (version 2.0, Roche Diagnostics; detection limit 500 IU/ml). When the quantitative assay showed undetectable levels of HCV RNA, a qualitative Amplicor HCV assay (version 2.0, Roche Diagnostics; detection limit 50 IU/ml) was applied. We defined patients that had undetectable HCV RNA at later than 24 weeks of treatment as ULVR.

Patient characteristics assessed at baseline included age, sex, body mass index (BMI), histological results of pre-treatment liver biopsy (Metavir scoring: F0 = no fibrosis; F1 = portal fibrosis; F2 = few septa; F3 = many septa without cirrhosis; F4 = cirrhosis), platelet count, serum HCV RNA before treatment, viral amino acid (aa) substitutions, and 2-log decrement in serum HCV RNA from baseline at 12 and 24 weeks of the treatment. Viral aa substitutions were determined for the IFN sensitivity-determining region (ISDR) in the HCV NS5A and substitutions at positions 70 and 91 in the HCV core region, as previously described [5–7]. The ISDR was defined as wild type when there were 1 or no aa substitutions, and defined as mutant type when there were 2 or more.

Results

Overall Response in Patients with HCV Genotype 1 and High Viral Load

The SVR rate for combination treatment in all the patients with genotype 1 and high viral load was 41.5% (54/130) by intention-to-treat analysis, and 54.3% (51/94) by per-protocol-study analysis.

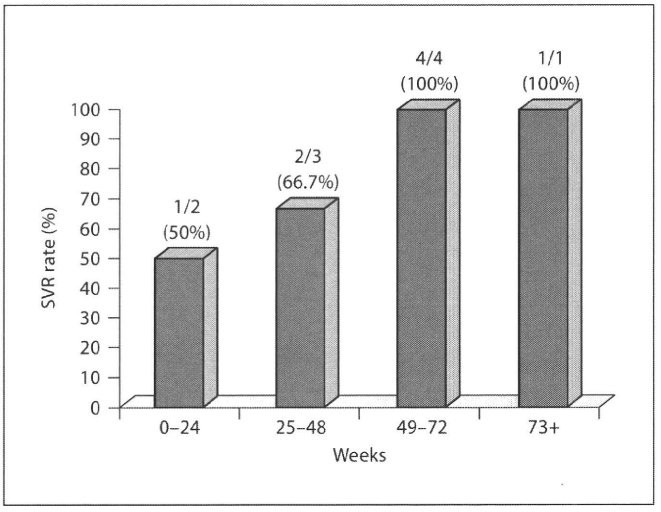

Fig. 1. The duration of maintaining undetectable HCV RNA while undergoing PEG-IFN/RBV combination treatment (maintenance treatment) was correlated with SVR rate. The horizontal axis represents the duration between the time serum HCV RNA was undetectable and the termination of the treatment.

Efficacy and Side Effects of Extended Treatment in ULVR

Among 10 ULVR, 8 patients achieved SVR by the extended treatment. The characteristics for ULVR are shown in table 1. Eight patients (80%) were older than 60 years of age and 7 (70%) were female. Four of the 8 patients with SVR showed severe fibrosis (F3) or cirrhosis (F4). Among 5 patients with SVR who were assessed for aa substitutions in HCV RNA, 3 patients showed a mutant type at ISDR and 4 showed wild type at both aa 70 and aa 91 in the HCV core region, while 2 patients with non-SVR showed substitutions at either aa 70 or aa 91. Six of 10 (60%) ULVR patients achieved a 2-log decrement of serum HCV RNA from baseline at 12 weeks and 8 patients (80%) at 24 weeks. Eight patients (80%) had undetectable HCV RNA within 36 weeks of treatment and the remaining 2 patients (1 each of the non-SVR and SVR groups) had undetectable HCV RNA at 48 and 60 weeks, respectively. The total duration of the combination treatment ranged from 59 to 119 weeks, with a mean duration of 80 weeks. All patients with SVR received extended treatment beyond 72 weeks with the exception of 1 patient, while treatment was stopped before 72 weeks for 2 patients with non-SVR.

Figure 1 shows the SVR rates according to the duration of maintaining undetectable serum HCV RNA while undergoing treatment (maintenance treatment). The SVR

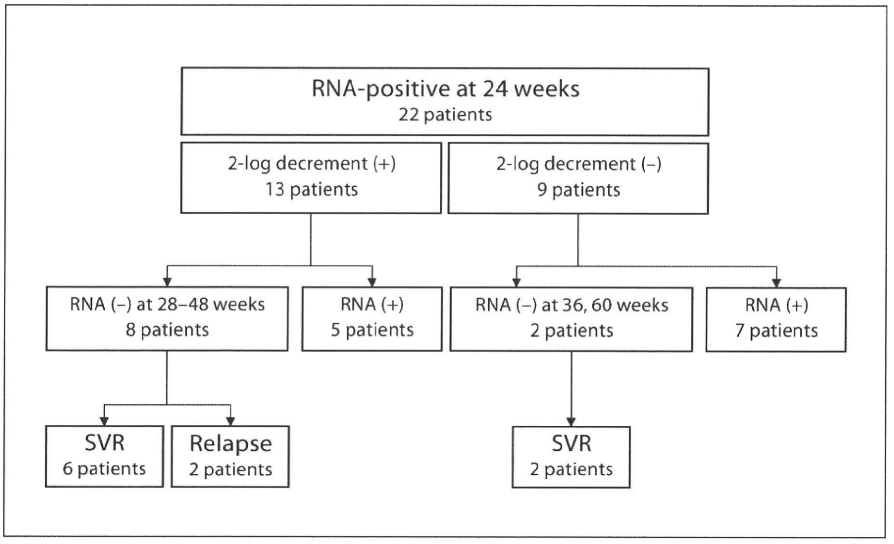

Fig. 2. Outcomes of the extended combination treatment in 22 patients who had detectable HCV RNA at 24 weeks of treatment. wks = Weeks.

Table 1. Patient characteristics of ULVR

Case No.	Age years	Gender	BMI	F stage	Platelet count/ μl	HCV RNA KIU/ml	ISDR	HCV core mutation		HCV RNA 2-log decrease		RNA negative weeks	Total treatment duration weeks	SVR
								aa 70	aa 91	12 weeks	24 weeks			
1	56	F	22.5	2	16.2	975	M	W	W	yes	yes	28	77	yes
2	64	F	26.7	3	18.9	2,400	W	W	W	yes	yes	28	83	yes
3	71	F	18.5	1	9.9	1,380	M	W	W	yes	yes	28	83	yes
4	77	M	23.7	4	7.8	166	ND	ND	ND	yes	yes	32	59	yes
5	62	F	19.6	1	23.6	252	M	W	W	–	yes	32	85	yes
6	63	F	19.9	3	8.9	895	ND	ND	ND	–	yes	32	119	yes
7	65	F	21.2	1	23.2	445	W	M	W	–	–	26	82	yes
8	60	F	22.1	3	13.6	1,350	ND	ND	ND	–	–	60	81	yes
9	73	M	20.2	1	8.2	943	M	M	W	yes	yes	48	62	–
10	64	M	26.6	1	16.8	1,750	W	W	M	yes	yes	32	68	–

M = mutant type; W = wild type; ND = not determined.

rate correlated well with the duration of the maintenance treatment. All 5 ULVR achieved SVR when maintenance treatment was continued for longer than 48 weeks.

The PEG-IFN dose was reduced in 4 of 10 (40%) ULVR within 8 weeks of the treatment, and RBV dose was reduced in 3 of 10 ULVR within 28 weeks of the treatment. Dose reduction of both PEG-IFN and RBV was required for 2 patients. Dose reduction was not required during the extended treatment duration and none of the treatments were terminated because of side effects.

Response to the Extended Treatment in Patients with Detectable HCV RNA at 24 Weeks

The outcomes of the extended combination treatment for 22 patients, including 10 ULVR, who had detectable HCV RNA at 24 weeks, are shown in figure 2. Among 13 patients who achieved a 2-log decrement in serum HCV RNA at 24 weeks, eight patients achieved undetectable HCV RNA between 28 and 48 weeks, including 6 with SVR. Five patients remained positive for serum HCV RNA despite extended treatment for 55–105 weeks with a mean duration of 88 weeks. Even in 9 patients who did not achieve a 2-log decrement in HCV RNA at 24 weeks,

2 patients (22%) achieved SVR as a result of extended treatment beyond 72 weeks, while the remaining 7 patients did not.

Discussion

For the treatment of patients infected with HCV genotype 1 and who have high viral load, a 72-week course of PEG-IFN and RBV treatment has become a standard treatment regimen for late virological responders [3, 4]. However, the benefit of extended treatment in patients who had undetectable serum HCV RNA later than 24 weeks of the treatment remains to be elucidated. In the present study, the extended combination treatment attained 80% (8/10) SVR in ULVR and 46% (6/13) SVR in patients who achieved 2-log decrement in HCV RNA from baseline at 24 weeks but still had detectable HCV RNA. Furthermore, here we describe 2 cases with SVR that failed to achieve a 2-log decrement in serum HCV RNA from baseline at 24 weeks.

Several host and viral factors contribute to SVR in PEG-IFN and RBV combination treatment for Japanese patients infected with HCV genotype 1 and who have high viral load. The host factors include younger age, male gender, mild liver fibrosis, platelet count, LDL cholesterol values and γ-glutamyl transpeptidase values. The viral factors include aa substitutions in the ISDR of HCV NS5A and aa substitutions in the HCV core region [5–9]. In addition, the total dose of PEG-IFN or RBV is another important factor that can affect the treatment outcome [8, 9]. In the present study, most ULVR were difficult-to-treat patients with respect to host factors, 8 patients (80%) were older than 60 years of age and 7 patients (70%) were female. Furthermore, 4 of 8 patients with SVR (50%) showed severe fibrosis (F3) or cirrhosis (F4) which is another well-known refractory factor to IFN treatment. These patient characteristics were significantly different from those of patients who achieved early virological response, having undetectable HCV RNA within 12 weeks of the treatment (data not shown). On the other hand, ULVR with SVR had positive viral factors regarding the aa substitutions in the ISDR and HCV core regions. Although we cannot exclude the possibility that these viral factors contributed to SVR rate even in ULVR, an extended combination treatment regimen is still considered a feasible treatment strategy for ULVR.

There are several limitations to this study. First, the Amplicor HCV assay method was used to measure serum HCV RNA in this study. The results presented here are likely to be different if patients were monitored by TaqMan qPCR, a state-of-the-art method with higher sensitivity. Second, this study is retrospective and the duration and dose of the treatment varied considerably. Further prospective studies are necessary to confirm an optimal treatment regimen for ULVR.

In conclusion, the extended duration of PEG-IFN and RBV combination treatment is beneficial in terms of virological response, even for ULVR. The extension of the treatment does not seem to increase side effects or the rate of dose reductions, and treatment should be continued until the serum HCV RNA remains undetectable for at least 24 weeks and, if possible, for longer than 48 weeks during the course of treatment.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

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

2 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: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.

3 Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Page GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S: 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.

4 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;46:1688–1694.

5 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C: Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96:224–230.
6 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996; 334:77–81.
7 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H: 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 2005;48:372–380.
8 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: 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 2007;46:403–410.
9 Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyoda J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H: 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 2009;44:952–963.