Table 2. Results of Univariate and Multivariate Analyses of Factors Associated With Survival of Patients Treated by Surgical Resection

Variable	No.	% 5-Year Survival Rate	Hazard Ratio (95% CI)	*P*
Univariate analysis				
No. of tumors				.012
Single	167	82.4	–	
Multiple	32	59.8	–	
Albumin, g/dL				.020
≥3.8	134	91.1	–	
<3.8	64	73.1	–	
Prothrombin time (%)				.003
≥80	168	83.0	–	
<80	29	60.1	–	
Multivariate analysis				
Prothrombin time, %, ≥80/<80			2.72 (1.56-4.74)	<.001

CI indicates confidence interval.

Table 3. Results of Univariate and Multivariate Analyses of Factors Associated With Survival in Patients Treated With Radiofrequency Ablation

Variable	No.	% 5-Year Survival Rate	Hazard Ratio (95% CI)	*P*
Univariate analysis				
Platelets, $\times 10^4/mm^3$				.006
≥10	111	84.1	–	
<10	98	65.5	–	
DCP, AU/mL				<.001
<100	187	77.3	–	
≥100	13	33.6	–	
Multivariate analysis				
DCP, AU/L, <100/≥100			5.49 (2.23-13.5)	<.001
Platelets, $\times 10^4/mm^3$, ≥10/<1			2.70 (1.24-5.88)	.012

CI indicates confidence interval; DCP, des-gamma-carboxy prothrombin; AU, arbitrary units.

Factors Associated With Survival in Patients in the RFA Group

We also evaluated the factors associated with the survival of 209 patients treated with RFA (Table 3). Platelet count $\geq 1.0 \times 10^5$ and DCP <100 arbitrary units (AU)/L were significant in univariate analysis, whereas 12 other variables were not associated with survival. Multivariate analysis identified DCP <100 AU/L (HR, 5.49; 95% CI, 2.23-13.5; $P < .001$) and platelet count $\geq 1.0 \times 10^5$ (HR, 2.70; 95% CI, 1.24-5.88; $P = .012$) as significant and independent determinants of survival.

Factors Associated With Recurrence-free Survival in Patients in the Resection Group

Next, we evaluated the factors associated with recurrence-free survival in patients treated with surgical resection (Table 4). Presence of a single tumor, serum albumin ≥3.8 g/dL, platelet count $\geq 1.0 \times 10^5$, and prothrombin time ≥80% were significant in the univariate analysis, whereas 10 other variables were not significant factors for recurrence-free survival. In multivariate analysis, single tumor (HR, 2.39; 95% CI, 1.51-3.80; $P < .001$), serum albumin ≥3.8 g/dL (HR, 1.54; 95% CI, 1.02-2.32;

Table 4. Results of Univariate and Multivariate Analyses of Factors Associated With Recurrence-free Survival Among Patients Treated With Resection

Variable	No.	% 3-Year Survival Rate	Hazard Ratio (95% CI)	P
Univariate analysis				
No. of tumors				<.001
Single	166	55.4	–	
Multiple	32	24.5	–	
Albumin, g/dL				.009
≥3.8	63	71.7	–	
<3.8	134	42.1	–	
Platelets, $\times 10^4/mm^3$				.025
≥10	137	60.3	–	
<10	60	33.0	–	
Prothrombin time, %				.009
≥80	167	55.7	–	
<80	29	29.0	–	
Multivariate analysis				
No. of tumors, single/multiple			2.39 (1.51-3.80)	<.001
Albumin, g/dL, ≥3.8/<3.8			1.54 (1.02-2.32)	.040
Platelets, $\times 10^4/mm^3$, ≥10/<10			1.47 (1.03-2.12)	.036

CI indicates confidence interval.

$P = .040$), and platelet count $\geq 1.0 \times 10^5$ (HR, 1.47; 95% CI, 1.03-2.12; $P = .036$) were independent prognostic factors for recurrence-free survival.

Factors Associated With Recurrence-free Survival in Patients in the RFA Group

Factors associated with recurrence-free survival were evaluated in patients treated by RFA. The 3-year recurrence-free survival rate was 44.7% in 185 patients with DCP <100AU/L, whereas it was 0.0% in 13 patients with DCP ≥100 AU/L. Univariate and multivariate analysis identified only DCP <100 AU/L (HR, 6.82; 95% CI, 3.49-13.3; $P < .001$) as a significant determinant of recurrence-free survival.

Survival and Recurrence-free Survival in Patients With DCP >100 AU/L

Figure 2 shows the cumulative survival rate, and Figure 3 shows the recurrence-free survival rate based on DCP levels. The survival rate and recurrence-free survival rate were associated with DCP in the RFA group, but they were not associated with DCP in the resection group. AFP >400 AU/L was associated with neither survival rate nor recurrence-free survival rate in either the resection or the RFA group. Therefore, 27 selected patients from the resection group and 13 from the RFA group whose DCP was ≥100 AU/L were examined to determine whether the overall survival rate was different between the resection and RFA groups. The backgrounds of the 2 groups based on treatment procedure are shown in Table 5. Treatment procedure (resection), age <65 years and serum albumin >3.8 g/dL were significant in the univariate analysis. Multivariate analysis revealed that treatment procedure (HR, 1.26; 95% CI, 1.04-1.53; $P = .020$) was a significant and independent determinant in the overall survival rate (Table 6).

DISCUSSION

Patients with HCC usually have a history of chronic liver disease, especially cirrhosis. Unfortunately, even when curative therapy is performed, tumor recurrence is frequent. For this reason, less invasive treatment procedures are needed to preserve liver function.

The Barcelona Clinic Liver Cancer (BCLC) guideline for the treatment of HCC recommends resection for patients with a single HCC and Child-Pugh A who have no other complications.[5] The suggested option for RFA includes patients with multiple tumors and associated

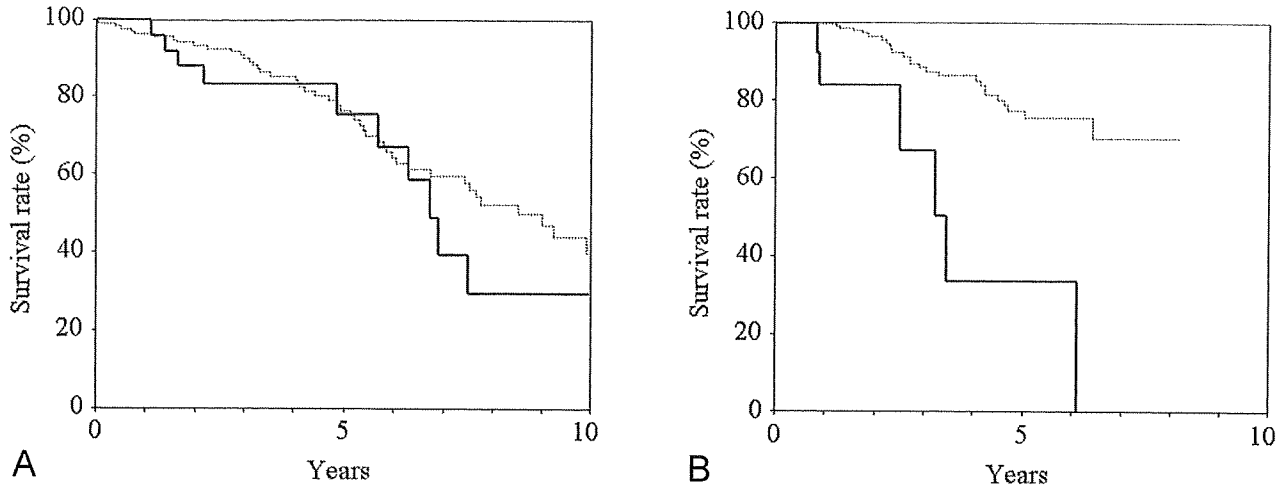

FIGURE 2. Cumulative overall survival rate of the patients who underwent surgical resection and radiofrequency ablation based on des-gamma-carboxy prothrombin (DCP) level is shown. Solid line indicates DCP level ≥100 AU/L; dotted line, DCP level <100 AU/L. (A) Cumulative survival rate of patients who underwent resection based on DCP level is shown. (B) Cumulative survival rate of patients who underwent radiofrequency ablation (RFA) based on DCP level is shown. Prognosis of patients who underwent RFA varied according to DCP level, whereas prognosis of patients who underwent resection was independent of DCP level.

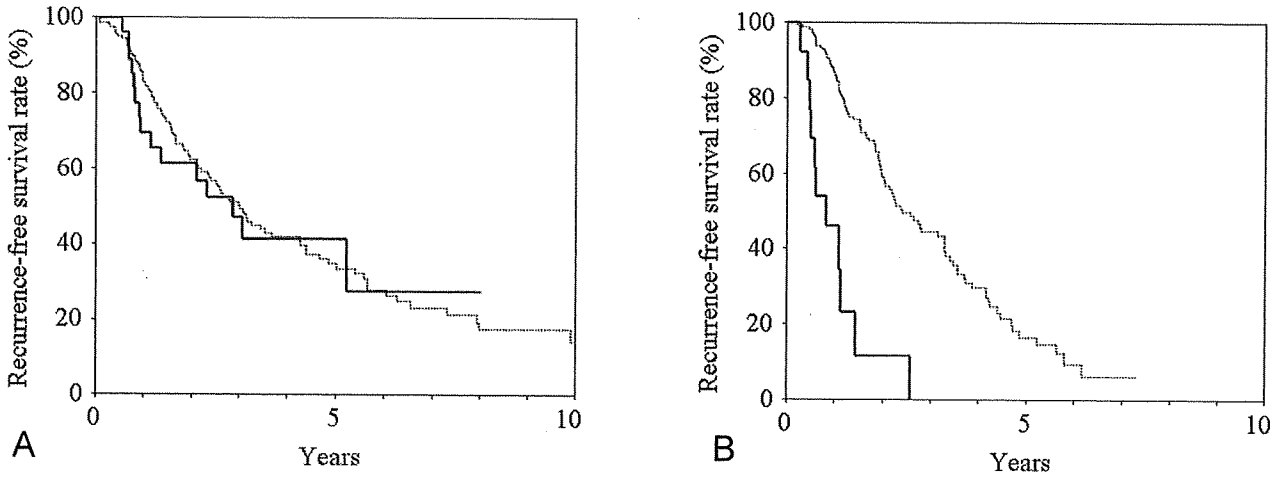

FIGURE 3. Cumulative recurrence-free survival (RFS) rate of the patients who underwent surgical resection and radiofrequency ablation based on des-gamma-carboxy prothrombin (DCP) level is shown. Solid line indicates DCP level ≥100 AU/L; dotted line, DCP level <100 AU/L. (A) Cumulative RFS rate of patients who underwent resection based on DCP level is shown. (B) Cumulative RFS rate of patients who underwent RFA based on DCP level is shown. Prognosis of patients who underwent RFA varied according to DCP level.

disease. However, in clinical practice, RFA is widely applied as a curative treatment for variable stages of HCC. Advances in imaging diagnosis have allowed identification of small HCC measuring <2 cm during the course of chronic liver disease.[3] The use of RFA seems to be an excellent option for the aforementioned tumors.

In the present study, we focused on the malignant potential of HCC and examined 2 representative tumor markers of HCC. AFP has been used as a tumor marker for HCC worldwide, and is considered by some as a predictor of survival or recurrence after RFA.[12] DCP is also useful as a prognostic factor in patients with HCC.[20-22]

In the present study, serum albumin levels and prothrombin time, which reflect liver function, were significantly associated with survival in patients who undergo resection similar to tumor multiplicity. Likewise, serum

Table 5. Clinical Background of 27 Patients Who Underwent Resection and 13 Patients Who Underwent Radiofrequency Ablation

Factors	Resection Group, n = 27	RFA Group, n = 13	*P*
Age, y*	60 (35-73)	67 (50-78)	.006
Sex (men:women)	23:4	10:3	NS
HBV:HCV:others	13:12:2	1:9:3	.015
Habitual alcohol intake, yes: no	3:24	2:11	NS
Diameter of HCC, mm*	22 (14-30)	22 (10-30)	NS
No. of HCC, single:multiple	26:1	9:4	.031
Tumor vascularity, present:absent	27:0	12:1	NS
Albumin, g/dL*	3.8 (3.3-4.3)	3.4 (2.6-4.1)	.003
Bilirubin, mg/dL*	0.9 (0.4-1.9)	0.9 (0.4-1.8)	NS
AST, IU/L*	38 (16-240)	49 (17-145)	NS
Platelets, $\times 10^4/mm^3$*	15.1 (6.0-24.5)	10.5 (4.5-24.6)	.025
Prothrombin time, %*	94 (79-112)	86 (73-110)	NS

*Data are expressed as median (range).

Table 6. Results of Univariate and Multivariate Analyses of Factors Associated With Survival Among Patients With Serum Des-gamma-carboxy Prothrombin Level ≥100

Variable	No.	% 5-Year Survival Rate	Hazard Ratio (95% CI)	*P*
Univariate analysis				
Age, y				.026
<65	25	87.7	—	
≥65	15	45.5	—	
Albumin, g/dL				.024
≥3.8	12	100	—	
<3.8	28	58.4	—	
Treatment procedure				.012
Resection	27	83.4	—	
RFA	13	33.6	—	
Multivariate analysis				
Treatment procedure				.020
Resection or RFA			1.26 (1.04-1.53)	

CI indicates confidence interval; RFA, radiofrequency ablation.

albumin level, platelet count, prothrombin time, and presence of multiple tumors were associated with recurrence-free survival. Alternatively, in RFA patients, in addition to platelet count, which indicates severity of portal hypertension, DCP levels were significant predictors of survival. Likewise, DCP levels were also significant predictors in recurrence-free survival. It is noteworthy that both survival and disease-free survival rates of patients who undergo RFA, but not resection, are correlated with DCP levels by multivariate analysis.

It is difficult to explain why DCP influenced survival and disease-free survival in the RFA group but not the resection group. We speculate that a high level of serum tumor marker reflects a high tumor malignant potential. Therefore, for a biologically aggressive tumor like HCC, resection is recommended over RFA because the radical nature of surgical resection may be superior to RFA.

According to previous reports, DCP is related to histological features of HCC.[21,22] Shirabe et al[21] examined

218 HCC patients who underwent surgical resection for HCC and concluded that serum DCP level is a predictor of microvascular invasion. They identified microvascular invasion in 44% of their patients with DCP ≥100 AU/L, but in only 16% of patients with DCP <100 AU/L. Shimada et al[22] examined explanted liver transplants and reported that serum DCP level is associated with vascular invasion and HCC recurrence. When we evaluated the relationship between clinicopathological features and serum DCP level in the resection group, microvascular invasion was found in 11 (44.0%) of 25 patients with DCP ≥100 AU/L, but in only 22 (13.6%) of 162 patients with DCP <100 AU/L, which was similar to the results in Shirabe et al.[21]

Unfortunately, in the RFA group, histological examination was performed only on nodules that showed atypical image findings. Moreover, it is sometimes difficult to judge microscopic vascular invasion in small specimens obtained by needle biopsy. However, we cannot deny the presence of microscopic vascular invasion in patients with high levels of tumor markers who have been treated with RFA. Furthermore, high levels of DCP are not only a marker of malignancy, but also indicate the biologic aggressiveness and progression of the HCC tumor. Hence, HCCs with high levels of DCP have greater chances of hypervascularity and early infiltration than do HCCs with lower levels of DCP.

In general, microscopic vascular invasion or intrahepatic metastasis is a poor prognostic factor for survival and recurrence-free survival even in patients who undergo surgical resection.[23-25] Why was survival and recurrence-free survival not different among the resection group in our study? One of the reasons is that we included patients with an HCC of a maximum diameter of ≤3 cm. In contrast, most previous studies included HCC as large as 5 cm in diameter.[23-25] The biological features of malignancy might be worse in such large tumors. We speculate that in HCC measuring ≤3 cm (median 2.0 cm), minimal microscopic vascular invasion or intrahepatic metastasis adjacent to the main tumor can be curatively resected by surgery, whereas these sometimes become incompletely necrotic even when a sufficient surrounding margin is obtained from treatment with RFA.

Analysis of factors associated with survival in patients with DCP of ≥100 AU/L showed that the type of treatment procedure (eg, hepatectomy) significantly influenced outcome. In contrast, no such relationship was found in patients with DCP of <100 AU/L. These results indicate that DCP is an important factor in selecting treatment procedure for patients with HCC measuring ≤3 cm and numbering ≤3 tumors.

In conclusion, DCP levels were significant predictors of both survival and recurrence-free survival in the RFA group. Hence, when the level of DCP is high, hepatic resection should be the treatment of choice even if the maximum tumor diameter is ≤3 cm and there are ≤3 tumors. If the level is low, RFA should be considered, because it is less invasive.

Because the current study was retrospective in nature, it has certain limitations and potential biases. The baseline characteristics of the 2 groups were quite different. Although we enrolled only Child-Pugh A patients, the resection group was younger and had better liver function. However, etiology of the liver disease was also different; the overall survival rates were not significantly based on etiology in either the resection or the RFA group. Therefore, we believe that the etiology of liver disease could be ignored in these patients. Our study did not uncover the reason for the high risk of mortality and tumor recurrence in patients in the RFA group with high levels of DCP. A cohort validation study is needed to confirm our results. In addition, clinicopathological and molecular analyses are also needed to define the biological significance of the biomarker.

Conflict of Interest Disclosures

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References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin.* 2005;55:74-108.
2. Kiyosawa K, Umemura T, Ichijo T, et al. Hepatocellular carcinoma: recent trend in Japan. *Gastroenterology.* 2004; 127:S17-S26.
3. Ikeda K, Saitoh S, Koida I, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology.* 1993;18:47-53.
4. Mazzaferro V, Regalia E, Doci R, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med.* 1996;334:693-699.

5. Bruix J, Sherman M. Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology.* 2005;42: 1208-1236.
6. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis.* 1999;19:329-338.
7. Cha C, DeMatteo RP, Blumgart LH. Surgery and ablative therapy for hepatocellular carcinoma. *J Clin Gastroenterol.* 2002;35(5 suppl 2):S130-S137.
8. Fan ST, Ng IO, Poon RT, Lo CM, Liu CL, Wong J. Hepatectomy for hepatocellular carcinoma: the surgeon's role in long-term survival. *Arch Surg.* 1999;134:1124-1130.
9. Chen MS, Li JQ, Zheng Y, et al. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg.* 2006;243:321-328.
10. Lu MD, Kuang M, Liang LJ, et al. Surgical resection versus percutaneous thermal ablation for early-stage hepatocellular carcinoma: a randomized clinical trial [in Chinese]. *Zhonghua Yi Xue Za Zhi.* 2006;86:801-805.
11. Lencioni R, Cioni D, Crocetti L, et al. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology.* 2005;234:961-967.
12. Choi D, Lim HK, Rhim H, et al. Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. *Eur Radiol.* 2007;17: 684-692.
13. Livraghi T, Meloni F, Di Stasi M, et al. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology.* 2008; 47:82-89.
14. Vauthey JN, Lauwers GY, Esnaola NF, et al. Simplified staging for hepatocellular carcinoma. *J Clin Oncol.* 2002; 20:1527-1536.
15. Liver Cancer Study Group of Japan. Classification of Primary Liver Cancer. First English Edition. Tokyo, Japan: Kanehara Press Co.; 1997.
16. Shimada M, Takenaka K, Fujiwara Y, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein positive status as a new prognostic indicator after hepatic resection for hepatocellular carcinoma. *Cancer.* 1996;78:2094-2100.
17. Imamura H, Matsuyama Y, Miyagawa Y, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. *Br J Surg.* 1999;86:1032-1038.
18. Kobayashi M, Ikeda K, Someya T, et al. Stepwise hook extension technique for radiofrequency ablation therapy of hepatocellular carcinoma. *Oncology.* 2002;63:139-144.
19. Cox DR. Regression models and life tables. *J R Stat Soc.* 1972;34:187-220.
20. Nagaoka S, Yatsuhashi H, Hamada H, et al. The des-gamma-carboxy prothrombin index is a new prognostic indicator for hepatocellular carcinoma. *Cancer.* 2003;98: 2671-2677.
21. Shirabe K, Itoh S, Yoshizumi T, et al. The predictors of microvascular invasion in candidates for liver transplantation with hepatocellular carcinoma-with special reference to the serum levels of des-gamma-carboxy prothrombin. *J Surg Oncol.* 2007;95:235-240.
22. Shimada M, Yonemura Y, Ijichi H, et al. Living donor liver transplantation for hepatocellular carcinoma: a special reference to a preoperative des-gamma-carboxy prothrombin value. *Transplant Proc.* 2005;37:1177-1179.
23. Sasaki A, Iwashita Y, Shibata K, Matsumoto T, Ohta M, Kitano S. Improved long-term survival after liver resection for hepatocellular carcinoma in the modern era: retrospective study from HCV-endemic areas. *World J Surg.* 2006;30:1567-1578.
24. Grazi GL, Cescon M, Ravaioli M, et al. Liver resection for hepatocellular carcinoma in cirrhotics and noncirrhotics. Evaluation of clinicopathologic features and comparison of risk factors for long-term survival and tumour recurrence in a single centre. *Aliment Pharmacol Ther.* 2003;17(suppl 2):119-129.
25. Hanazaki K, Kajikawa S, Koide N, Adachi W, Amano J. Prognostic factors after hepatic resection for hepatocellular carcinoma with hepatitis C viral infection: univariate and multivariate analysis. *Am J Gastroenterol.* 2001;96:1243-1250.

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An Open Pilot Study Exploring the Efficacy of Fluvastatin, Pegylated Interferon and Ribavirin in Patients with Hepatitis C Virus Genotype 1b in High Viral Loads

Hitomi Sezaki[a] Fumitaka Suzuki[a] Norio Akuta[a] Hiromi Yatsuji[a]
Tetsuya Hosaka[a] Masahiro Kobayashi[a] Yoshiyuki Suzuki[a] Yasuji Arase[a]
Kenji Ikeda[a] Yuzo Miyakawa[b] Hiromitsu Kumada[a]

[a]Department of Hepatology, Toranomon Hospital, and [b]Miyakawa Memorial Research Foundation, Tokyo, Japan

Key Words
Hepatitis, chronic · Virus, hepatitis C · Statin · Interferon, pegylated · Ribavirin

Abstract
Objective: Response to pegylated (PEG) interferon (IFN) and ribavirin is achieved only in 40–50% of patients infected with hepatitis C virus (HCV) of genotype 1 in high viral loads, which needs to be improved. ***Methods:*** In an open-label pilot study, fluvastatin (HMG-CoA reductase inhibitor), 20 mg daily, was given along with PEG-IFN/ribavirin to 21 patients with chronic hepatitis C. They were followed for HCV RNA in serum. ***Results:*** During treatment for 48 weeks, HCV RNA was lost from serum in 93% of the patients. In the 15 patients who received 48-week therapy, a sustained virological response (SVR) with loss of HCV RNA 24 weeks after completion was achieved in 10 (67%), including 7 of the 9 (78%) male and 3 of the 6 (50%) female patients. In the remaining 6 patients who received 72-week therapy, SVR was gained in 4 (67%), including 1 of the 2 male and 3 of the 4 female patients aged 56, 58 and 62 years, respectively. ***Conclusion:*** Fluvastatin could be used safely to increase the response to PEG-IFN and ribavirin, especially in aged women who respond poorly to combined PEG-IFN/ribavirin.

Introduction

Over the world approximately 190 million people are persistently infected with hepatitis C virus (HCV) [1], and about 30% of them develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC) [2]. Interferon (IFN) is the only drug that can resolve HCV infection. However, using the most advanced treatment currently available, with pegylated (PEG)-IFN and ribavirin for 24–48 weeks, a sustained virological response (SVR) defined by the loss of HCV RNA from serum 24 weeks after completion of therapy is achieved in 40–80% of the patients [3–5]. The response rate is influenced by host factors such as sex, age and ethnicity [6–8], as well as virological factors including genotypes and viral loads [9]. It remains unsatisfactorily low in patients infected with HCV genotype 1 at merely 40–50%, and dismal in women aged 50 years or older at merely 22% [10]. Hence, there is an imminent need to improve the efficacy of antiviral treatment for terminating HCV infection in these patients.

Efforts have been devoted toward increasing the efficacy to PEG-IFN/ribavirin therapy. Inhibitors of viral protease, alone or in combination with the IFN-based treatment, have been found effective in preliminary studies [11, 12]. Recently, drugs that can inhibit the key enzyme

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Hitomi Sezaki, MD
Department of Hepatology, Toranomon Hospital
Minato-ku
Tokyo 105-8470 (Japan)
Tel. +81 44 877 5111, Fax +81 44 860 1623, E-Mail hitomis@mx1.harmonix.ne.jp

for controlling the synthesis of cholesterol, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, have gained attention due to their potential to decrease the replication of HCV in clinical and experimental settings [13, 14].

More than three quarters of Japanese patients are infected with HCV genotype 1b in high viral loads. They are much older than Western patients owing to the widespread HCV infection which struck Japan about 20 years ago [15]. Owing to this, the response to PEG-IFN/ribavirin in Japanese patients with chronic hepatitis C is poorer than that in Western countries. In an open-label pilot study, 21 patients with chronic hepatitis C, who were infected with HCV-1b at high viral loads, received triple treatment with fluvastatin, PEG-IFN and ribavirin. On-treatment viral dynamics and SVR achieved by the triple therapy could offer hope for improving the response in patients infected with HCV-1 in high viral loads.

Patients and Methods

From December 2005 to March 2006, 21 patients with chronic hepatitis C agreed to receive fluvastatin, in addition to standard PEG-IFN/ribavirin treatment, at the Department of Hepatology, Toranomon Hospital, Metropolitan Tokyo. They all were: (1) positive for antibody to HCV (anti-HCV) and HCV RNA of genotype 1b, and not co-infected with HCV of the other genotypes; (2) negative for hepatitis B surface antigen or antibody to human immunodeficiency virus type-1 (HIV-1); (3) confirmed within the past 2 months to have high HCV RNA levels of ≥100 KIU/ml, which is the Japanese definition of high viral loads [16, 17]; (4) platelet counts >80 × 10^3/mm^3 and without cirrhosis diagnosed by ultrasonography; (5) body weight >40 kg and not pregnant or lactating; (6) a total alcohol intake of <500 kg during the past; (7) without HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; (8) without antiviral or immunosuppressive treatment during the previous 3 months, and (9) with the wish to comply to the treatment protocol for 48–72 weeks.

They were followed for liver function and virological markers at least monthly during treatment and until 24 weeks after completion of triple treatment. Informed consent was obtained from each patient, and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Markers of HCV Infection

Anti-HCV was determined by third-generation enzyme-linked immunosorbent assay (ELISA) using commercial kits (Ortho HCV Ab ELISA Test 3; Chiron Cooperation, Emeryville, Calif., USA). HCV RNA was determined quantitatively by polymerase chain reaction (PCR; Cobas Amplicor HCV Monitor ver. 2.0, Roche Diagnostics, Tokyo, Japan), with a dynamic range from 5 to 5,000 KIU/ml, in sera diluted 10-fold at baseline, as well as every 2 weeks until 8 weeks after initiation of therapy and monthly thereafter. Sera negative for HCV RNA (<5 KIU/ml) by the quantitative assay were tested by qualitative PCR (Amplicor, Roche Molecular Systems, Inc., Branchburg, N.J., USA) with the detection limit at 100 copies/ml.

Fluvastatin Added to Combined PEG-IFN and Ribavirin

Patients received subcutaneous PEG-IFN-α2b (PEG-Intron, Schering-Plough Corp., Kenilworth, N.J., USA) weekly at a median dose of 1.5 (range 1.3–1.7) μg/kg, along with oral ribavirin daily at a median dose of 12.3 (range 10.2–13.7) mg/kg for 48–72 weeks. The dose of ribavirin was adjusted according to body weight: 600 mg for patients weighing ≤60 kg; 800 mg for those between >60 and <80 kg, and 1,000 mg for those ≥80 kg. In addition, the patients received 20 mg oral fluvastatin daily for 48–72 weeks.

Results

Patients Who Received Triple Treatment with Fluvastatin, PEG-IFN and Ribavirin

The baseline characteristics of the 21 patients infected with HCV-1b at a high viral load and who received triple therapy with fluvastatin, PEG-IFN and ribavirin are listed in table 1. Their median age was 56 years, and 11 (52%) were men. Their hematological and biochemical values including serum lipids were within normal limits, except for elevated levels of AST and ALT. A lack of mutations affecting the response to IFN in the core protein, i.e., mutations at positions 70 and 91 [16], and amino acid substitutions in the IFN-sensitivity determining region [18] were detected in HCV RNA from 29 and 35% of the patients.

Loss of HCV RNA from Serum in Patients with Triple Treatment

Figure 1 illustrates the cumulative loss of HCV RNA from the serum in 21 patients infected with HCV-1b at high loads. HCV RNA was cleared in 52% of the patients during the first 12 weeks. It decreased slowly thereafter, and was lost in 93% of the patients at 48 weeks. Triple treatment was continued in 6 of the 21 patients for an additional 24 weeks. HCV RNA was cleared from the serum in 5 (83%) of them at completion of the 72-week treatment.

Sustained Virological Response in Patients Receiving Triple Treatment

Of the 21 patients given triple treatment, 19 received it for 48 weeks. Skin rashes and general malaise developed in 2 of them at weeks 17 and 24, respectively, and fluvastatin was withdrawn while PEG-IFN and ribavirin were continued. Six patients continued to receive triple treatment for an additional 24 weeks. Therefore, the loss of HCV RNA from the serum 24 weeks after completion of

Table 1. Baseline characteristics of patients (n = 21) with chronic hepatitis C who received triple treatment with fluvastatin, PEG-IFN and ribavirin

	Normal ranges	Patients
Age, years	NA	56 (32–63)
Men	NA	11 (52%)
Albumin, g/dl	3.9–5.2	3.8 (3.4–4.2)
Hemoglobin, g/dl	11.3–17.0	14.4 (12.1–16.4)
Platelets, $\times 10^3/mm^3$	141–350	190 (119–240)
AST, IU/l	13–33	50 (21–208)
ALT, IU/l	6–42	64 (23–391)
γ-GTP	9–109	36 (11–301)
ICG_{15}, %	<10	12 (2–27)
HCV RNA, KIU/ml	NA	2,000 (14– >5,000)
Past treatments	NA	8 (36%)
Ribavirin dose, mg/kg	NA	12.3 (10.2–13.7)
Serum lipids		
Total cholesterol, mg/dl	122–240	163 (124–273)
LDL-C, mg/dl	86–160	99 (59–187)
HDL-C, mg/dl	35–75	46 (28–73)
Mutations in HCV RNA		
Wild-type at aa70	NA	12 (57%)
Wild-type at aa91	NA	10 (48%)
Double wild, aa71/aa90	NA	6 (29%)
Mutations in ISDR	NA	6/17 (35%)

Data are expressed as the number of patients with percentages in parentheses or median values with ranges in parentheses. AST = Aspartate aminotransferase; ALT = alanine aminotransferase; γ-GTP = γ-glutamyltranspeptidase; ICG_{15} = retention of indocyanine green at 15 min; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; NA = not applicable.

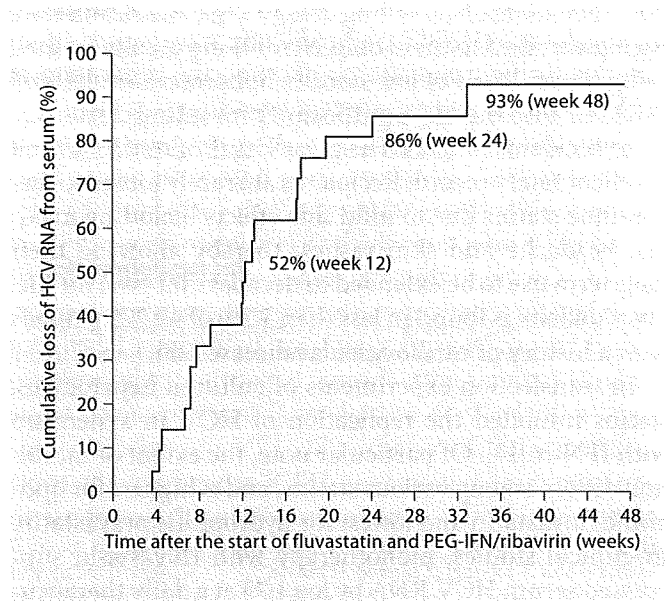

Fig. 1. Kaplan-Meier table illustrating the cumulative loss of HCV RNA from the serum in patients with chronic hepatitis C. The patients received fluvastatin in addition to PEG-IFN/ribavirin and were followed during 48 weeks of treatment.

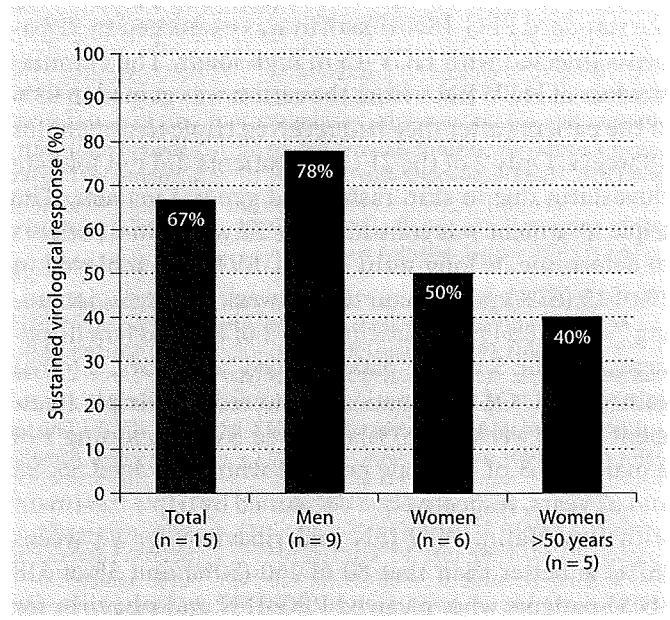

Fig. 2. Sustained virological response in patients with chronic hepatitis C to the combined treatment with fluvastatin, PEG-IFN and ribavirin for 48 weeks. SVR was compared among the total, male and female patients, as well as the female patients aged more than 50 years.

therapy was assessed in the remaining 15 patients. Figure 2 shows the SVR in 15 patients. SVR was accomplished in 10 (67%) of them, including 7 of 9 (78%) male patients and 3 of 6 (50%) female patients. Of the 5 female patients aged >50 years, 2 (40%) gained SVR.

Of the 6 patients who received triple treatment for 72 weeks, SVR was accomplished in 4 (67%), including 1 of 2 male and 3 of 4 female patients who were aged 56, 58 and 62 years, respectively.

Discussion

Statins comprise a group of drugs capable of inhibiting HMG-CoA reductase and can regulate the synthesis of cholesterol by competing with the authentic substrate [19,

20]. They are the best selling drugs in the world, and have been prescribed to more than 30 million patients for lowering serum levels of low-density lipoprotein cholesterol (LDL-C) with the main purpose of preventing cardiovascular accidents [21, 22]. The great virtue of statins is their excellent safety record. Recipients are rarely forced to discontinue statins due to mild side effects including myalgia, headache and skin rashes, thereby allowing their long-term use to be extended to decades and even for life. Liver toxicity is found in less than 1.2% of 49,275 patients with a history of cardiovascular disease [23].

In transfection experiments of cultured hepatocytes, statins inhibited the replication of HCV in synergism with IFN-α [14]. Of particular note, the extent of inhibition differs among various statins, and is highest for lipophilic fluvastatin and lowest for hydrophilic pravastatin. In clinical studies, monotherapy with fluvastatin suppressed serum HCV RNA by log 1.75 at a daily therapeutic dose of <80 mg [13], while no such effects were gained with 20 mg atorvastatin daily [24]. Hence, the ability to inhibit HCV replication may differ among diverse statins through mechanisms not directly associated with lowered levels of LDL-C; fluvastatin decreases total cholesterol and LDL-C levels to a lesser extent than the other statins in current use.

In this open-label pilot study, the response to triple treatment with fluvastatin 20 mg daily supplementing the standard PEG-IFN/ribavirin was examined in 21 patients infected with HCV-1b in high loads. The cumulative loss of HCV RNA from the serum was gained in 93% of the patients after they had received triple treatment for 48 weeks. Only 2 of the 21 (10%) patients did not tolerate fluvastatin due to skin rashes and general malaise. The triple treatment was continued for 72 weeks in 6 patients to determine its long-term effects. SVR was achieved in 10 of 15 (67%) patients on the 48-week schedule, including 7 of 9 (78%) male patients and 3 of 6 (50%) female patients. Of the 5 female patients aged >50 years, 2 (40%) gained SVR. Of the 6 patients who received triple treatment for 72 weeks, 4 (67%) achieved SVR, including 1 of 2 male and 3 of 4 female patients who were aged 56, 58 and 62 years, respectively. SVR gained by triple treatment with fluvastatin, PEG-IFN and ribavirin for 48 weeks (67%) is better than that 80 of 160 (50%) and 55 of 118 (47%) patients who received PEG-IFN and ribavirin for 48 weeks [16, 25]; they all were infected with HCV-1b at high loads.

Sex and age are the two principal host factors influencing the response to IFN-based treatment. Although women respond better to combined IFN and ribavirin than men in large-scale multinational studies in Western countries [8, 26], target patients were rather young with the median age of about 40–41 years. Sex-dependent responsiveness to IFN is reversed with age, however, and the response to PEG-IFN/ribavirin is much poorer in female than male patients aged 50 years or older (22/100 [22%] vs. 50/94 [53%], $p < 0.001$) [10]. In view of the estradiols that can prohibit fibrosis in experimental cirrhosis induced in rats [27], levels of female sex hormones, which are lower in women than men older than 50 years [28], could be responsible for the sex difference in response to IFN of patients with chronic hepatitis C. Supplementation of aged female patients with estradiols may increase the response, but it can accelerate physiological osteoporosis [29, 30]. Since statin therapy does not increase the risk of severe hepatotoxicity in patients with chronic hepatitis C [31], fluvastatin may provide aged women with an advantage in gaining SVR to PEG-IFN/ribavirin. Furthermore, fluvastatin does not augment the side effects of other drugs, such as cyclosporine A and warfarin, because it is metabolized by CYP2C9 in the liver, unlike other statins that are disposed by CYP3A4 as other drugs [32].

Should statins interfere with the replication of HCV, it remains an open question how they do it. At least three mechanisms may be conceived for the decrease in HCV replication in patients receiving statins. HCV circulates in association with LDL-C [33], and on that basis, the LDL-C receptor has been proposed for infection of hepatocytes [34–36]. HCV RNA replicates in association with lipid droplets in hepatocytes [37, 38], and therefore, the synthesis of cholesterol blocked by statins can reduce the basis on which HCV thrives. However, these two scenarios seem to stand at odds with the response to IFN being better in patients with higher LDL-C levels [39, 40]. Statins prohibit the synthesis of mevalonate that is modified into geranylgeranyl (containing 20 carbons) and then farnesyl (15 carbons) [19]. They both prenylate most cellular proteins to make them lipophilic toward associations with membranes expressing their biological activities [41]. As an extension to these, the geranylgeranylation of host proteins, which may be required for the replication of HCV, is implicated in the capacity of statins to downregulate HCV infection [42–44]. For that matter, most pleiotropic effects of statins, including a deceased incidence of dementia and Alzheimer disease [45–47], could be associated with the inhibition of prenylation rather than lowered levels of LDL-C in the serum [48].

This study is not without limitations. The number of patients receiving fluvastatin along with PEG-IFN and ribavirin is small and fails to gain statistical power in any comparison with those given PEG-IFN/ribavirin in previous studies. The ability of other statins in improving the response to PEG-IFN/ribavirin has not been examined to discover the mechanism of how fluvastatin improves the response to antiviral treatments. The promising results obtained in this study hopefully will invite further interest for planning clinical trials with extended numbers of patients and targeting elderly women in particular. Meanwhile, it may be worth looking back in the database to examine the response of patients receiving PEG-IFN/ribavirin for chronic hepatitis C, simultaneously with statins for controlling LDL-C levels, to see if they fared better than those not taking statins routinely.

Acknowledgment

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References

1 Cohen J: The scientific challenge of hepatitis C. Science 1999;285:26–30.
2 Seeff LB: Natural history of chronic hepatitis C. Hepatology 2002;36:S35–S46.
3 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.
4 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: Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–355.
5 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.
6 Kanwal F, Hoang T, Spiegel BM, Eisen S, Dominitz JA, Gifford A, Goetz M, Asch SM: Predictors of treatment in patients with chronic hepatitis C infection – role of patient versus nonpatient factors. Hepatology 2007;46:1741–1749.
7 Poynard T, Bedossa P, Opolon P: Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349:825–832.
8 Conjeevaram HS, Fried MW, Jeffers LJ, Terrault NA, Wiley-Lucas TE, Afdhal N, Brown RS, Belle SH, Hoofnagle JH, Kleiner DE, Howell CD: Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. Gastroenterology 2006;131:470–477.
9 Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, Hashimoto M, Iwasaki S, Kobayashi M, Hiromitsu K: Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. Hepatology 1994;19:1088–1094.
10 Sezaki H, Suzuki F, Kawamura Y, Yatuji H, Hosaka T, Akuta N, Kobayashi M, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Miyakawa Y, Kumada H: Poor response to pegylated interferon and ribavirin in aged women infected with hepatitis C virus of genotype 1b in high viral loads. Dig Dis Sci, in press.
11 Lawitz E, Rodriguez-Torres M, Muir AJ, Kieffer TL, McNair L, Khunvichai A, McHutchison JG: Antiviral effects and safety of telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in hepatitis C patients. J Hepatol 2008;49:163–169.
12 Forestier N, Reesink HW, Weegink CJ, McNair L, Kieffer TL, Chu HM, Purdy S, Jansen PL, Zeuzem S: Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with hepatitis C. Hepatology 2007;46:640–648.
13 Bader T, Fazili J, Madhoun M, Aston C, Hughes D, Rizvi S, Seres K, Hasan M: Fluvastatin inhibits hepatitis C replication in humans. Am J Gastroenterol 2008;103:1383–1389.
14 Ikeda M, Abe K, Yamada M, Dansako H, Naka K, Kato N: Different anti-HCV profiles of statins and their potential for combination therapy with interferon. Hepatology 2006;44:117–125.
15 Yoshizawa H, Tanaka J, Miyakawa Y: National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. Intervirology 2006;49:7–17.
16 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686–1695.
17 Sezaki H, Suzuki F, Kawamura Y, Yatsuji H, Hosaka T, Akuta N, Kobayashi M, Suzuki Y, Arase Y, Ikeda K, Kumada H: Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. Hepatol Res 2007;37:787–792.
18 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.
19 Goldstein JL, Brown MS: Regulation of the mevalonate pathway. Nature 1990;343:425–430.
20 Istvan ES, Deisenhofer J: Structural mechanism for statin inhibition of HMG-CoA reductase. Science 2001;292:1160–1164.
21 Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM: Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med 2004;350:1495–1504.
22 Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ: Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. J Am Coll Cardiol 2004;44:720–732.
23 de Denus S, Spinler SA, Miller K, Peterson AM: Statins and liver toxicity: a meta-analysis. Pharmacotherapy 2004;24:584–591.

24 O'Leary JG, Chan JL, McMahon CM, Chung RT: Atorvastatin does not exhibit antiviral activity against HCV at conventional doses: a pilot clinical trial. Hepatology 2007;45: 895–898.
25 Akuta N, Suzuki F, 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 polymorphisms in the viral core protein and very early dynamics of viremia. Intervirology 2007;50:361–368.
26 Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J: Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426–1432.
27 Yasuda M, Shimizu I, Shiba M, Ito S: Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. Hepatology 1999;29:719–727.
28 Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL: Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab 1998; 83:2266–2274.
29 Need AG, O'Loughlin PD, Morris HA, Horowitz M, Nordin BE: The effects of age and other variables on serum parathyroid hormone in postmenopausal women attending an osteoporosis center. J Clin Endocrinol Metab 2004;89:1646–1649.
30 Siris ES, Brenneman SK, Barrett-Connor E, Miller PD, Sajjan S, Berger ML, Chen YT: The effect of age and bone mineral density on the absolute, excess, and relative risk of fracture in postmenopausal women aged 50–99: results from the National Osteoporosis Risk Assessment (NORA). Osteoporos Int 2006;17:565–574.
31 Khorashadi S, Hasson NK, Cheung RC: Incidence of statin hepatotoxicity in patients with hepatitis C. Clin Gastroenterol Hepatol 2006;4:902–907.
32 Corsini A, Bellosta S, Baetta R, Fumagalli R, Paoletti R, Bernini F: New insights into the pharmacodynamic and pharmacokinetic properties of statins. Pharmacol Ther 1999; 84:413–428.
33 Andre P, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, Pol S, Brechot C, Paranhos-Baccala G, Lotteau V: Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. J Virol 2002;76:6919–6928.
34 Molina S, Castet V, Fournier-Wirth C, Pichard-Garcia L, Avner R, Harats D, Roitelman J, Barbaras R, Graber P, Ghersa P, Smolarsky M, Funaro A, Malavasi F, Larrey D, Coste J, Fabre JM, Sa-Cunha A, Maurel P: The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. J Hepatol 2007;46:411–419.
35 Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX: Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. Proc Natl Acad Sci USA 1999;96:12766–12771.
36 Monazahian M, Bohme I, Bonk S, Koch A, Scholz C, Grethe S, Thomssen R: Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol 1999;57:223–229.
37 Aizaki H, Lee KJ, Sung VM, Ishiko H, Lai MM: Characterization of the hepatitis C virus RNA replication complex associated with lipid rafts. Virology 2004;324:450–461.
38 Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, Bartenschlager R, Wakita T, Hijikata M, Shimotohno K: The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol 2007;9:1089–1097.
39 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.
40 Gopal K, Johnson TC, Gopal S, Walfish A, Bang CT, Suwandhi P, Pena-Sahdala HN, Clain DJ, Bodenheimer HC Jr, Min AD: Correlation between beta-lipoprotein levels and outcome of hepatitis C treatment. Hepatology 2006;44:335–340.
41 Zhang FL, Casey PJ: Protein prenylation: molecular mechanisms and functional consequences. Annu Rev Biochem 1996;65:241–269.
42 Kapadia SB, Chisari FV: Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. Proc Natl Acad Sci USA 2005;102:2561–2566.
43 Wang C, Gale M Jr, Keller BC, Huang H, Brown MS, Goldstein JL, Ye J: Identification of FBL2 as a geranylgeranylated cellular protein required for hepatitis C virus RNA replication. Mol Cell 2005;18:425–434.
44 Ye J, Wang C, Sumpter R Jr, Brown MS, Goldstein JL, Gale M Jr: Disruption of hepatitis C virus RNA replication through inhibition of host protein geranylgeranylation. Proc Natl Acad Sci USA 2003;100:15865–15870.
45 Wolozin B, Kellman W, Ruosseau P, Celesia GG, Siegel G: Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors. Arch Neurol 2000;57:1439–1443.
46 Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA: Statins and the risk of dementia. Lancet 2000;356:1627–1631.
47 Vaughan CJ: Prevention of stroke and dementia with statins: effects beyond lipid lowering. Am J Cardiol 2003;91:23B–29B.
48 Liao JK, Laufs U: Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol 2005;45:89–118.

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ORIGINAL ARTICLE

Poor Response to Pegylated Interferon and Ribavirin in Older Women Infected with Hepatitis C Virus of Genotype 1b in High Viral Loads

Hitomi Sezaki · Fumitaka Suzuki · Yusuke Kawamura · Hiromi Yatsuji · Tetsuya Hosaka · Norio Akuta · Masahiro Kobayashi · Yoshiyuki Suzuki · Satoshi Saitoh · Yasuji Arase · Kenji Ikeda · Yuzo Miyakawa · Hiromitsu Kumada

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Abstract *Background* Response to treatment in patients with chronic hepatitis C, with reference to age and gender, has not been examined fully. *Aim* The influence of gender and age on treatment with pegylated interferon (PEG-IFN) and ribavirin was evaluated in a retrospective study. *Methods* PEG-IFN and ribavirin were given for 48 weeks to 179 men and 121 women infected with hepatitis C virus (HCV) of genotype 1b in high viral loads (>100 kIU/ml). *Results* Sustained virological response at 24 weeks after treatment was poorer in women than men who were aged ≥50 years (22% vs 53%, $P < 0.001$). Among the patients aged ≥50 years who had received ≥80% of the doses of PEG-IFN, ribavirin, or both, women responded less often than men (26% vs 64%, $P < 0.001$; 33% vs 61%, $P = 0.022$; and 32% vs 63%, $P = 0.016$; respectively). In multivariate analysis, male gender, retention of indocyanine green, ribavirin dose and compliance with therapy increased sustained virological response. *Conclusions* Response to combined PEG-IFN and ribavirin is poorer in female than male patients with hepatitis C who are aged ≥50 years, irrespective of compliance with treatment. Low estrogen levels in older women could be responsible for their impaired response to PEG-IFN and ribavirin.

H. Sezaki (✉) · F. Suzuki · Y. Kawamura · H. Yatsuji · T. Hosaka · N. Akuta · M. Kobayashi · Y. Suzuki · S. Saitoh · Y. Arase · K. Ikeda · H. Kumada
Department of Hepatology, Toranomon Hospital, Minato-ku, Tokyo 105-8470, Japan
e-mail: hitomis@mx1.harmonix.ne.jp

Y. Miyakawa
Miyakawa Memorial Research Foundation, Tokyo, Japan

Keywords Aging · Women · Chronic hepatitis C · Genotypes · Interferon · Ribavirin

Introduction

There are an estimated 170 million people worldwide that are chronically infected with hepatitis C virus (HCV) [1]. HCV can persist in 70–80% of individuals who have been exposed to it, and it can induce chronic liver disease, through cirrhosis to hepatocellular carcinoma (HCC) in approximately 30% of them until 30–40 years after they were infected [2–4]. A number of viral and host factors influence the velocity of fibrosis progression in chronic hepatitis C. Thus, stage and grade of hepatitis are more severe in patients who are infected with HCV genotype 1 in high viral loads [5–7]. Male gender, age and intake of alcohol accelerate fibrosis, as well [8–10].

Interferon (IFN) combined with ribavirin has been the most effective and favored treatment of chronic hepatitis C to date. The combined treatment with the standard IFN can terminate HCV-1 infection with high viral loads in approximately 20% [11], and that with pegylated IFN (PEG-IFN) in >40% [12]. Owing to hemolytic side effects, however, women are less tolerant to ribavirin [13]. Although the response to combined treatment has been shown to be better in women than in men in previous studies, there remains a possibility that it could be influenced by age. Hence, there is a need for the comparison of the response between men and women in different age groups.

Virological response to PEG-IFN and ribavirin at the end of a 48-week treatment (ETR), as well as sustained virological response (SVR) 24 weeks after the completion of therapy, was compared between 179 men and 121

women who were infected with HCV-1b in high viral loads. In patients aged ≥50 years, both ETR and SVR were poorer in women than in men, irrespective of the total dose of IFN, ribavirin or both.

Methods

Study Population

From December 2001 to February 2006, 490 consecutive patients with chronic hepatitis C received combination therapy with PEG-IFN and ribavirin at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. The following inclusion criteria were met by 300 (61%) patients: they were (1) positive test results for antibodies to HCV (anti-HCV) and for HCV RNA genotype 1b by qualitative methods, and not co-infected with HCV of other genotypes; (2) negative test results for hepatitis B surface antigen or antibodies to human immunodeficiency virus type-1 (HIV-1); (3) confirmed findings of high HCV RNA levels ≥100 kIU/ml, which is the Japanese definition of high viral loads [14, 15], within the past 2 months; (4) no cirrhosis diagnosed by laparoscopy and ultrasonography, and with platelet counts $>80 \times 10^3/mm^3$; (5) body weight ≥40 kg and not pregnant or lactating; (6) total alcohol intake <500 kg in the past; (7) no HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; (8) no treatment with antivirals or immunosuppressants during the previous 3 months; and (9) with the wish to comply with the treatment protocol for 48 weeks. None of them received growth factors before or during the study period.

The 300 patients, comprising 179 men and 121 women, received PEG-IFN and ribavirin for 48 weeks and were followed for at least 24 weeks after completion of this combination therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Serum Markers of HCV Infection

Anti-HCV was determined by third-generation enzyme-linked immunosorbent assay (ELISA) by commercial kits (Ortho HCV Ab ELISA Test 3; Chiron Cooperation, Emeryville, CA, USA). HCV RNA was determined quantitatively by polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor ver. 2.0, Roche Diagnostics, Tokyo, Japan) in serum diluted tenfold at the baseline, as well as at least monthly during and after treatment; it has a dynamic range between 5 kIU/ml and 5,000 kIU/ml. Sera negative for HCV RNA (<5 kIU/ml) by quantitative assay were tested by qualitative PCR (Amplicor, Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a detection limit at 100 copies/ml.

Combined PEG-IFN and Ribavirin Therapy

Patients underwent subcutaneous administration of PEG-IFN-α2b (PEG-Intron, Schering-Plough Corp, Kenilworth, NJ, USA), weekly, at a median dose of 1.4 μg/kg (range 0.8–1.9 μg/kg), together with ribavirin orally, at a median daily dose of 11 mg/kg (range 3.7–14.2 mg/kg) for 48 weeks. The dose of ribavirin was adjusted by body weight: 600 mg for patients weighing ≤60 kg; 800 mg for those between >60 kg and <80 kg; and 1,000 mg for those ≥80 kg. It was tapered in the 99 (33%) patients in whom hemoglobin levels decreased below 10 g/dl during the combination therapy.

Statistical Analysis

Variables were compared between groups by the chi-square test, Fisher's exact probability test and the Mann–Whitney U test. Differences in the loss of HCV RNA from the serum between groups was evaluated with the Kaplan–Meier life table with use of the log rank test. The influence of various factors on the response to PEG-IFN/ribavirin was evaluated by logistic regression in univariate and multivariate analyses. Analysis of all data was performed with the computer program SPSS software (SPSS Inc., Chicago, IL, USA), and a P value less than 0.05 was considered significant.

Results

Baseline Characteristics of Male and Female Patients Infected with HCV-1b in High Loads

PEG-IFN and ribavirin were given for 48 weeks to 179 men and 121 women who had been infected with HCV-1b in high viral loads (>100 kIU/ml). Table 1 compares baseline characteristics between them. Women were older, had lower hemoglobin values and platelet counts, and lower levels of albumin, gamma-glutamyl-transpeptidase (γ-GTP) and ferritin, than men. The stage of fibrosis was lower in women than in men, although their alanine aminotransferase (ALT) levels were comparable. Three months before the start of combination therapy, IFN had been given to 75 (42%) of the 179 male patients, comparably frequently to 40 of the 121 (33%) female patients. Age distribution for men and women is shown in Fig. 1. The proportion of patients ≥60 years was higher in women than in men (39% vs 19%, $P < 0.001$).

Table 1 Baseline characteristics of 300 patients with chronic hepatitis with high-titers of HCV-1b RNA who had received PEG-IFN and ribavirin for 48 weeks and were followed for 48 weeks or longer

Characteristic[a]	Men (n = 179)	Women (n = 121)	Differences P
Age (years)	50 (19–66)	57 (30–69)	<0.001
Previous IFN treatment	75 (42%)	40 (33%)	0.146
Hemoglobin (g/dl)	15.2 (11.5–17.8)	13.5 (11.2–15.1)	<0.001
Platelets ($\times 10^3$/mm^3)	176 (88–366)	165 (91–331)	0.025
Albumin (g/dl)	3.9 (3.2–4.6)	3.8 (3.0–4.6)	0.004
ALT (IU/l)	77 (23–504)	68 (19–391)	0.078
γ-GTP (IU/l)	78 (14–409)	37 (11–171)	0.011
LDL (mg/dl)	98 (50–176)	99 (57–168)	0.920
Ferritin (mg/l)	186 (<10–1,327)	95 (<10–4 42)	<0.001
ICG_{15} (%)	14 (4–41)	13 (2–31)	0.969
Stage (F0-1/F2-3)	80/66 (50 unknown)	42/55 (57 unknown)	0.050

ALT alanine aminotransferase, *γ-GTP* gamma-glutamyl transpeptidase, *LDL* low density lipoprotein, ICG_{15}, retention of indocyanine green at 15 min

[a] The means (ranges) are given

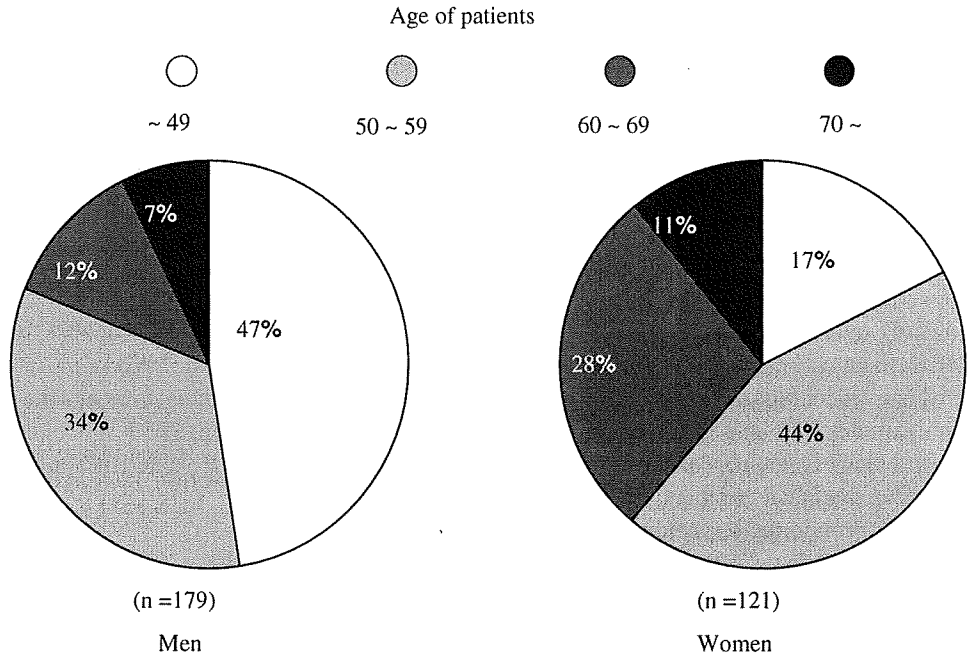

Fig. 1 Distribution of ages in the male and female patients with chronic hepatitis C who were infected with HCV-1b in high loads

Virological Response During the 48-Week Treatment with PEG-IFN and Ribavirin

On-treatment response to the combined treatment is compared between men and women in Fig. 2. Through 48 weeks on treatment, women gained a virological response less frequently than did men. ETR was achieved by 58% of women as against 83% of men ($P < 0.001$). Restricted to the patients who gained ETR, women lost HCV RNA from serum later than men did [median (range) 24.1 (2.0–36.4) vs 13.0 (2.0–48.0) weeks, $P < 0.001$]. Figure 3 depicts the on-treatment virological response in patients <50 years and those ≥50 years separately. The virological response was no different between men and women <50 years. However, it was poorer in women than

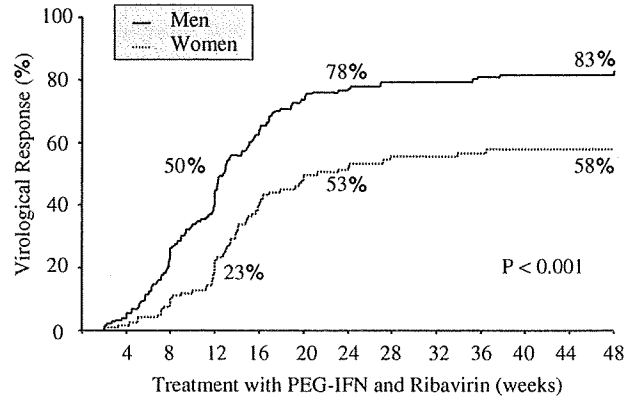

Fig. 2 On-treatment virological responses to PEG-IFN and ribavirin in male and female patients infected with HCV-1b in high viral loads

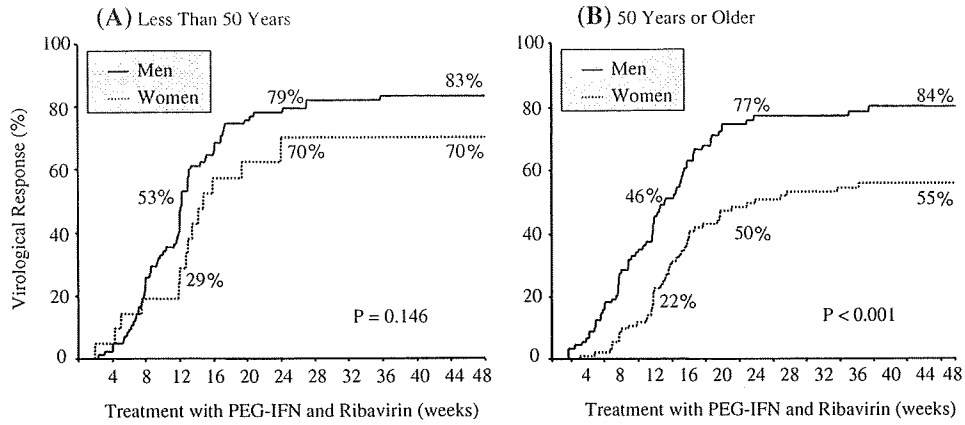

Fig. 3 On-treatment virological responses to combined IFN and ribavirin in male and female patients infected with HCV-1b in high viral loads who were less than 50 years (**a**) or 50 years or older (**b**)

in men ≥50 years. Differences between men and women in total (Fig. 2), therefore, were attributed to a poorer response of women ≥50 years to the combined treatment.

Sustained Virological Response to the 48-Week Treatment with PEG-IFN and Ribavirin

Sustained virological response 24 weeks after the completion of combined treatment was accomplished much less frequently in women than in men [33/121 (27%) vs 105/179 (59%), $P < 0.001$]. SVR was influenced by age both in men and in women (Fig. 4). It was found significantly less often in women than men who were 50 years or older.

Figure 5 illustrates the relationship between the earliest on-treatment virological response and SVR in men and women. Patients with a virological response at 4 weeks gained SVR invariably. However, in the patients with virological response in later weeks, SVR was achieved less frequently in women than in men. In the patients who had lost HCV RNA from the serum at 12 weeks, in particular, SVR was achieved significantly less often in women than in men (63% vs 88%, $P = 0.012$). The relationship

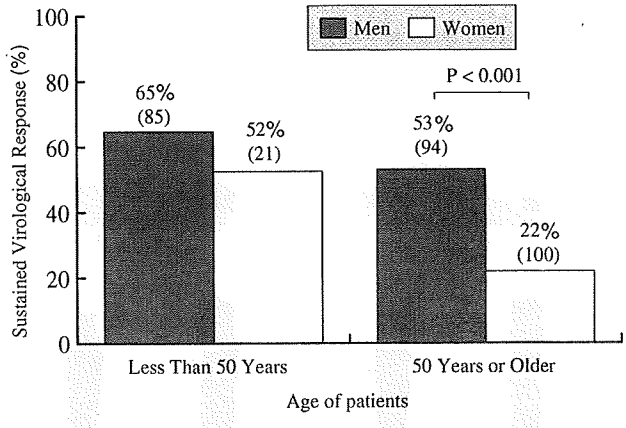

Fig. 4 Sustained virological response to PEG-IFN and ribavirin in male and female patients stratified by age. The number of patients is indicated in parentheses in each column

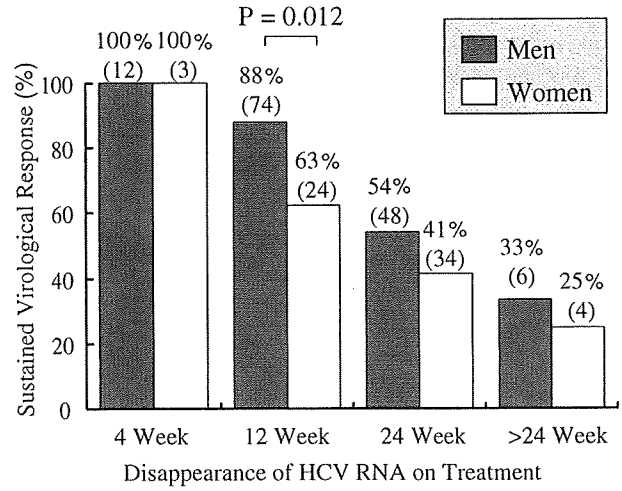

Fig. 5 Sustained virological response in male and female patients who lost HCV RNA from the serum at various weeks on treatment with PEG-IFN and ribavirin

between on-treatment virological response and SVR was compared among women in different age groups (Fig. 6). In the patients with the earliest virological response at 12 weeks and 24 weeks, SVR was achieved less frequently in women aged ≥50 years than in those <50 years, but the difference fell short of being significant due to the small numbers of patients in the comparison.

SVR and Compliance with PEG-IFN Therapy, Ribavirin Therapy, or Both

Table 2 compares compliance with the combined treatment between men and women. Either or both of PEG-IFN and ribavirin were tolerated to a lesser extent by women than by men. Thus, doses ≥80% were reached less frequently in women than in men for PEG-IFN or ribavirin, or both. The initial dose of ribavirin was no different between men and women.

SVR was achieved less frequently in women than in men who had received ≥80% of the dose of PEG-IFN

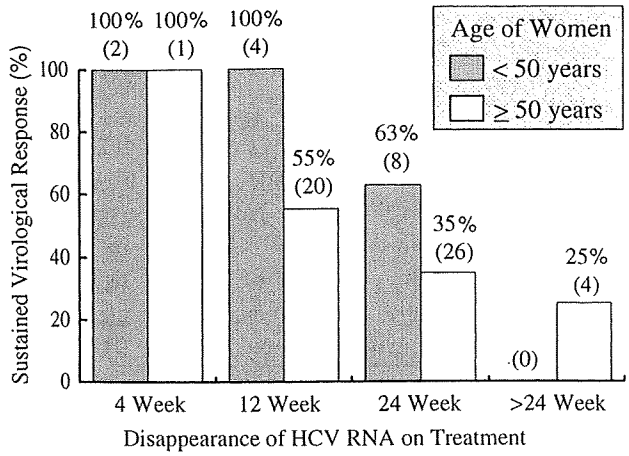

Fig. 6 Sustained virological response to PEG-IFN and ribavirin in female patients stratified by age who lost HCV RNA from serum at various weeks on treatment

Table 2 PEG-IFN and ribavirin received by patients with chronic hepatitis with high-titers of HCV-1b RNA

PEG-IFN and ribavirin	Men (n = 179)	Women (n = 121)	Differences P
Initial ribavirin dose (mg/kg body weight)	11.1 (5.0–14.1)	11.2 (3.7–14.3)	0.735
Total dose			
PEG-IFN ≥ 80%	139 (78%)	94 (53%)	<0.001
Ribavirin ≥ 80%	117 (65%)	42 (35%)	<0.001
Both ≥ 80%	110 (61%)	32 (27%)	<0.001
Withdrawn	28 (16%)	28 (23%)	0.131

[20/64 (31%) vs 92/138 (67%), $P < 0.001$], ribavirin [19/42 (45%) vs 79/120 (66%), $P = 0.027$] or both [13/32 (41%) vs 72/109 (66%), $P = 0.013$]. Again, differences were observed only in patients ≥50 years (Fig. 7). In the patients <50 years, in contrast, the rate of SVR was no different between women and men who had received ≥80% of the dose of PEG-IFN, ribavirin, or both.

The influence of age was compared between male and female patients in different age groups. SVR was achieved significantly more frequently in the men aged ≥60 years [88/145 (61%) vs 17/74 (37%), $P = 0.001$] and <60 years [17/34 (50%) vs 6/48 (13%), $P < 0.001$]. Likewise, SVR was more common in male than female patients aged 50–59 years [33/60 (55%) vs 16/53 (30%), $P = 0.013$].

Multivariate Analysis for Factors Accelerating the Response to PEG-IFN and Ribavirin Therapy

In univariate analysis, age, gender, hemoglobin, albumin, ICG_{15}, ribavirin dose and compliance with PEG-IFN therapy, ribavirin therapy, or both, influenced SVR. In multivariate analysis, only male gender, ICG_{15}, ribavirin dose and compliance with PEG-IFN, as well as both PEG-IFN and ribavirin, accelerated the chance of SVR (Table 3).

Discussion

In a retrospective study, response to PEG-IFN and ribavirin for 48 weeks was compared between 179 men and 121 women with chronic hepatitis C who had been infected with HCV-1b in high viral loads by the Japanese definition (>100 kIU/ml) [14, 15]. Loss of HCV RNA from serum occurred less often in women than in men throughout the 48 weeks of treatment. Both ETR (55% vs 83%, $P < 0.001$) and SVR (27% vs 59%, $P < 0.001$) were achieved significantly less frequently in women than in men. The observed low response to PEG-IFN and ribavirin stands at odds with the better response to antiviral treatments and slow progression of fibrosis in women than in men [9, 16, 17]. There are, however, viral and host factors other than gender that can influence the course of chronic hepatitis C and, by inference, the response to antiviral treatments.

Viral factors such as HCV genotypes and infection load affect the course of chronic hepatitis C. Thus, hepatitis is

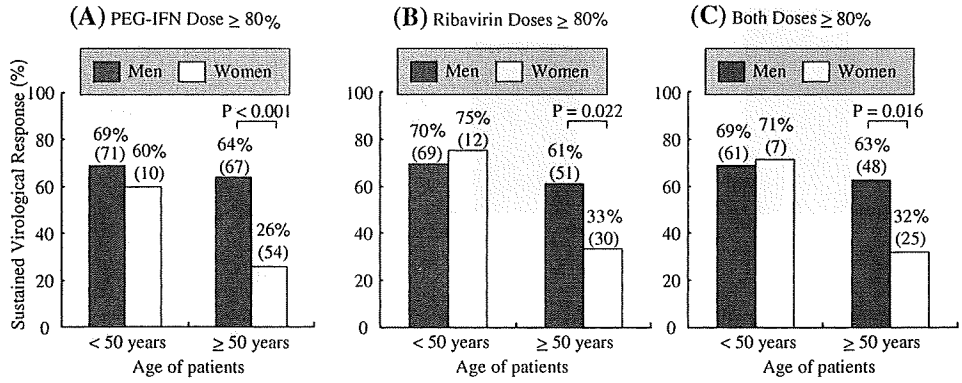

Fig. 7 Sustained virological response to PEG-IFN and ribavirin in patients who had received 80% or more of the dose of IFN (**a**), ribavirin (**b**) or both of them (**c**). Results are shown for men and women in two age groups

Table 3 Factors promoting the response to PEG-IFN and ribavirin in multivariate analysis

Factors	Odds ratio	95% Confidence interval	*P*
Male gender	3.50	1.71–7.17	0.001
$ICG_{15} \leq 13.5\%$	2.09	1.07–4.08	0.031
Ribavirin $\geq$ 11.1 mg/kg per day	2.17	1.11–4.25	0.024
Total PEG-IFN $\geq$ 80%	6.96	2.26–21.4	0.001
PEG-IFN/ribavirin $\geq$ 80%	12.66	2.32–71.4	0.003

more severe and less responsive to IFN in patients infected with HCV genotypes 1 and 4 than in those with HCV genotypes 2, 3 and 6 [18–22]. Likewise, high viral loads are associated with rapid progression of liver disease and poor response to IFN [23–25]. In our study, such viral factors were excluded in comparing the response to PEG-IFN and ribavirin between women and men. All the patients were infected with HCV genotype 1b in high viral loads (>100 kIU/ml).

Age influences the severity of chronic hepatitis C [9, 26], and disease progresses faster and response to antiviral therapy is poorer in older patients [23]. There were significant differences in age between female and male patients in our study. The women were older than the men [mean (range) 57 (30–69) years vs 50 (19–66) years, $P < 0.001$], and the proportion of patients $\geq$60 years was higher in women than in men (39% vs 19%, $P < 0.001$). Hence, the response to PEG-IFN and ribavirin was evaluated in patients aged $\geq$50 years and <50 years separately. There were no differences in the response between female and male patients <50 years, during and at the end of the 48-week treatment, as well as 24 weeks thereafter. However, ETR (55% vs 84%, $P < 0.001$) and SVR (22% vs 53%, $P < 0.001$) were gained significantly less often in women than men who were aged $\geq$50 years.

The influence of gender was observed, also, in patients aged $\geq$60 years and those aged 50–60 years. Hence, women would become less responsive than men to PEG-IFN and ribavirin after they had entered their fifties.

From a therapeutic notion, compliance with treatment can alter the response. Since ribavirin accumulates in erythrocytes and induces hemolysis, it is less tolerated in women who tend to be anemic than men without such an inclination [27]. At the baseline, women had lower levels of hemoglobin and ferritin than men. These would have been responsible for the lower tolerance to PEG-IFN and ribavirin in women than men in our study. In fact, $\geq$80% of the dose of PEG-IFN, ribavirin, or both, was tolerated less frequently in women than men ($P < 0.001$ for each). Even in the patients who had received $\geq$80% of the dose, however, the response to PEG-IFN and ribavirin was gained less frequently in women than in men. Again, the difference was due to a significantly lower response in female patients than in male patients aged $\geq$50 years, while the response was no different between those <50 years of age.

Taken altogether, the poorer response to PEG-IFN and ribavirin in women than in men was attributable to impaired response in the female patients aged $\geq$50 years. Older women with chronic hepatitis C, therefore, would be less responsive to the combined treatment with PEG-IFN and ribavirin currently in use. In support of this view, the response to human lymphoblastoid IFN for 24 weeks is dependent on gender and age [28]. The greatest physiological change precipitated in women by aging is a decreased serum concentration of bioavailable estrogen after they enter the menopause [29]. Estrogen has been shown to have an antifibrotic potential in both experimental and clinical studies. In experimental cirrhosis induced by dimethylnitrosamine in rats, administration of neutralizing antibodies to estradiol and ovariectomy enhanced fibrogenesis in female rats [30]. Hepatocytes have the receptor to estrogen [31], and myofibroblastic transformation in hepatic stellate cells of rats is inhibited in culture supplemented with this hormone [32]. Consequently, hepatic fibrosis progresses faster in menopausal women with chronic hepatitis C, and hormone replacement therapy may be able to prevent it [33]. Furthermore, in women aged $\geq$50 years, the number of estrogen receptor in hepatocytes decreases to one-half of that in those aged <50 years. This would stand in further support of the notion that the antifibrotic effects of decreased estrogen levels in patients aged $\geq$50 years with chronic hepatitis C would produce a lesser response to PEG-IFN and ribavirin.

Favorable effects of female sex hormones on hepatitis have long been suggested. Chronic hepatitis C is mild in menstruating women [34]; its activity is suppressed during pregnancy and enhanced after delivery [35]. The velocity of fibrosis progression is extremely low in young women exposed to HCV through mass-administration of immunoglobulin-D. Only two of 184 (1.2%) and four of 1,018 (0.4%) developed cirrhosis over 24 years and 20 years, respectively, in Irish and German studies [36, 37]. It does need to be pointed out, however, that the majority of women in those studies had not been followed beyond the menopause. There is a possibility that chronic hepatitis C may progress at a faster speed during their next few decades. Continued observations of them would be necessary to evaluate the validity of such an assumption.

Although decreased levels of estrogen can explain the enhanced activity of chronic hepatitis C in older women, as well as their concomitant resistance to PEG-IFN and ribavirin, it does not give an account of the better response in men than women who were aged $\geq$50 years. Feminization represented by gynecomastia is common in men

who have developed cirrhosis, and it can increase even in healthy men with age [38]. Possibly in the background of this phenomenon, circulating levels of free estrogen in men exceed those in women, after they enter their fifties, with margins widening with age [29]. It is tempting to speculate that elevated estrogen levels in men with chronic hepatitis C are responsible for their better response to the combination therapy than women who were aged ≥50 years. Whether or not such a speculation would hold would have to be evaluated by a comparison of estrogen levels between older men and women with chronic hepatitis C.

Although osteoporosis is an extrahepatic manifestation of chronic hepatitis C [39], hormone replacement therapy has been withheld for fear of potential hepatotoxicity. There is evidence, however, that oral contraceptives inhibit the progression of fibrosis in women [33]. It may lead to the possibility that the response to antiviral treatment in older women with chronic hepatitis C would be improve by substituting estrogen in them. The merit of hormone replacement therapy for them, of course, would need to be balanced against any harmful effects associated with it.

There are limitations in this study. All the patients were infected with genotype 1b in high viral loads. Hence, the results obtained may or may not be extended to patients with chronic hepatitis C who are infected with HCV of other genotypes in low viral loads. The influence of sex hormones needs to be substantiated by their determination in correlation with SVR. These limitations notwithstanding, the results obtained warrant a special caution in the treatment of women older than 50 years due to their lesser responsiveness to PEG-IFN and ribavirin.

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References

1. Cohen J. The scientific challenge of hepatitis C. *Science*. 1999;285:26–30. doi:10.1126/science.285.5424.26.
2. Alberti A, Chemello L, Benvegnu L. Natural history of hepatitis C. *J Hepatol*. 1999;31:S17–S24. doi:10.1016/S0168-8278(99)80369-9.
3. Alter HJ, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis*. 2000;20:17–35. doi:10.1055/s-2000-9505.
4. Seeff LB. Natural history of chronic hepatitis C. *Hepatology*. 2002;36:S35–S46. doi:10.1002/hep.1840360706.
5. Feray C, Gigou M, Samuel D, et al. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. *Gastroenterology*. 1995;108:1088–1096. doi:10.1016/0016-5085(95)90207-4.
6. Garcia-Samaniego J, Soriano V, Castilla J, et al. Influence of hepatitis C virus genotypes and HIV infection on histological severity of chronic hepatitis C. *Am J Gastroenterol*. 1997;92:1130–1134.
7. Pageaux GP, Ducos J, Mondain AM, et al. Hepatitis C virus genotypes and quantitation of serum hepatitis C virus RNA in liver transplant recipients: relationship with severity of histological recurrence and implications in the pathogenesis of HCV infection. *Liver Transpl Surg*. 1997;3:501–505. doi:10.1002/lt.500030504.
8. Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med*. 1999;340:1228–1233. doi:10.1056/NEJM199904223401602.
9. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet*. 1997;349:825–832. doi:10.1016/S0140-6736(96)07642-8.
10. Vogt M, Lang T, Frosner G, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. *N Engl J Med*. 1999;341:866–870. doi:10.1056/NEJM199909163411202.
11. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med*. 1998;339:1485–1492. doi:10.1056/NEJM199811193392101.
12. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med*. 2002;347:975–982. doi:10.1056/NEJMoa020047.
13. Hung CH, Lee CM, Lu SN, et al. Anemia associated with antiviral therapy in chronic hepatitis C: incidence, risk factors, and impact on treatment response. *Liver Int*. 2006;26:1079–1086. doi:10.1111/j.1478-3231.2006.01354.x.
14. Akuta N, Suzuki F, Kawamura Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol*. 2007;79:1686–1695. doi:10.1002/jmv.20979.
15. Sezaki H, Suzuki F, Kawamura Y, et al. Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatol Res*. 2007;37:787–792. doi:10.1111/j.1872-034X.2007.00132.x.
16. Conjeevaram HS, Fried MW, Jeffers LJ, et al. Peginterferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology*. 2006;131:470–477. doi:10.1053/j.gastro.2006.06.008.
17. Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet*. 1998;352:1426–1432. doi:10.1016/S0140-6736(98)07124-4.
18. Tsubota A, Chayama K, Ikeda K, et al. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. *Hepatology*. 1994;19:1088–1094.
19. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med*. 2004;140:346–355.
20. Hui CK, Yuen MF, Sablon E, Chan AO, Wong BC, Lai CL. Interferon and ribavirin therapy for chronic hepatitis C virus genotype 6: a comparison with genotype 1. *J Infect Dis*. 2003;187:1071–1074. doi:10.1086/368217.
21. Legrand-Abravanel F, Nicot F, Boulestin A, et al. Pegylated interferon and ribavirin therapy for chronic hepatitis C virus genotype 4 infection. *J Med Virol*. 2005;77:66–69. doi:10.1002/jmv.20414.
22. Yuen MF, Lai CL. Response to combined interferon and ribavirin is better in patients infected with hepatitis C virus genotype 6

than genotype 1 in Hong Kong. *Intervirology*. 2006;49:96–98. doi:10.1159/000087270.
23. Kumada T, Toyoda H, Honda T, et al. Treatment of chronic hepatitis C with interferon alone or combined with ribavirin in Japan. *Intervirology*. 2006;49:112–118. doi:10.1159/000087273.
24. Liang TJ, Rehermann B, Seeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann Intern Med*. 2000;132:296–305.
25. Trepo C. Genotype and viral load as prognostic indicators in the treatment of hepatitis C. *J Viral Hepat*. 2000;7:250–257. doi: 10.1046/j.1365-2893.2000.00233.x.
26. Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. *Gut*. 2002;51:248–252. doi:10.1136/gut.51.2.248.
27. Takaki S, Tsubota A, Hosaka T, et al. Factors contributing to ribavirin dose reduction due to anemia during interferon alfa2b and ribavirin combination therapy for chronic hepatitis C. *J Gastroenterol*. 2004;39:668–673. doi:10.1007/s00535-003-1363-9.
28. Hayashi J, Kishihara Y, Ueno K, et al. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. *Arch Intern Med*. 1998;158:177–181. doi: 10.1001/archinte.158.2.177.
29. Khosla S, Melton LJ 3rd, Atkinson EJ, O'Fallon WM, Klee GG, Riggs BL. Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. *J Clin Endocrinol Metab*. 1998;83:2266–2274. doi:10.1210/jc.83.7.2266.
30. Yasuda M, Shimizu I, Shiba M, Ito S. Suppressive effects of estradiol on dimethylnitrosamine-induced fibrosis of the liver in rats. *Hepatology*. 1999;29:719–727. doi:10.1002/hep.510290307.
31. Rossini GP, Baldini GM, Villa E, Manenti F. Characterization of estrogen receptor from human liver. *Gastroenterology*. 1989;96:1102–1109.
32. Shiba M, Shimizu I, Yasuda M, Ii K, Ito S. Expression of type I and type III collagens during the course of dimethylnitrosamine-induced hepatic fibrosis in rats. *Liver*. 1998;18:196–204.
33. Di Martino V, Lebray P, Myers RP, et al. Progression of liver fibrosis in women infected with hepatitis C: long-term benefit of estrogen exposure. *Hepatology*. 2004;40:1426–1433. doi: 10.1002/hep.20463.
34. Sartori M, Andorno S, Rigamonti C, Grossini E, Nicosia G, Boldorini R. Chronic hepatitis C is mild in menstruating women. *J Gastroenterol Hepatol*. 2000;15:1411–1417. doi:10.1046/j.1440-1746.2000.02368.x.
35. Latt NC, Spencer JD, Beeby PJ, et al. Hepatitis C in injecting drug-using women during and after pregnancy. *J Gastroenterol Hepatol*. 2000;15:175–181. doi:10.1046/j.1440-1746.2000.02060.x.
36. Levine RA, Sanderson SO, Ploutz-Snyder R, et al. Assessment of fibrosis progression in untreated Irish women with chronic hepatitis C contracted from immunoglobulin anti-D. *Clin Gastroenterol Hepatol*. 2006;4:1271–1277. doi:10.1016/j.cgh.2006.05.028.
37. Wiese M, Berr F, Lafrenz M, Porst H, Oesen U. Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in Germany: a 20-year multicenter study. *Hepatology*. 2000; 32:91–96. doi:10.1053/jhep.2000.8169.
38. Braunstein GD. Gynecomastia. *N Engl J Med*. 1993;328:490–495. doi:10.1056/NEJM199302183280708.
39. Carey EJ, Balan V, Kremers WK, Hay JE. Osteopenia and osteoporosis in patients with end-stage liver disease caused by hepatitis C and alcoholic liver disease: not just a cholestatic problem. *Liver Transpl*. 2003;9:1166–1173. doi:10.1053/jlts.2003.50242.