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Virological and Biochemical Features in Elderly HCV Patients with Hepatocellular Carcinoma: Amino Acid Substitutions in HCV Core Region as Predictor of Mortality after First Treatment

Suguru Ogura[a] Norio Akuta[a] Miharu Hirakawa[a] Yusuke Kawamura[a] Hiromi Yatsuji[a] Hitomi Sezaki[a] Tetsuya Hosaka[a] Masahiro Kobayashi[a] Fumitaka Suzuki[a] Yoshiyuki Suzuki[a] Mariko Kobayashi[b] Satoshi Saitoh[a] Yasuji Arase[a] Kenji Ikeda[a] Hiromitsu Kumada[a]

[a]Department of Hepatology and [b]Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan

Key Words
Hepatitis C virus · Core region · Hepatocellular carcinoma · Mortality

Abstract
Aims: We showed previously that amino acid (aa) substitutions in HCV genotype 1b (HCV-1b) core region are negative predictors of virological response to peginterferon + ribavirin therapy, and also risk factors of hepatocarcinogenesis. The aim of this study was to evaluate the impact of core aa substitutions on mortality in elderly patients. ***Methods:*** We compared the characteristics and survival of 92 elderly (≥75 years) patients with HCV-related hepatocellular carcinoma (HCC) (including 62 patients with HCV-1b) with those of 44 younger patients (<50 years, 34 patients with HCV-1b). ***Results:*** For all patients, univariate analysis identified female sex, history of blood transfusion, preserved liver function and glucose metabolism as significant variables in the elderly patients. In patients with HCV-1b-related HCC, univariate analysis identified preserved lipid metabolism as significant variable in addition to significant variables in overall patients. In elderly patients with HCV-1b-related HCC, multivariate analysis identified male sex, methionine of core aa91, and non-radical therapy as factors that influenced mortality after first treatment for HCC. ***Conclusions:*** Our results characterized elderly patients who develop HCC after HCV-1b infection, and suggested that aa substitutions of HCV-1b core region correlate with mortality of patients after first treatment for HCC.

Introduction

Hepatitis C virus (HCV) usually causes infection, which can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1–6]. In patients with HCV-chronic hepatitis, treatment with interferon (IFN) can induce viral clearance and marked biochemical and histological improvement [7, 8].

In Japan, the number of elderly HCC patients with HCV infection has increased in recent years, especially the proportion of female patients with chronic hepatitis [9–13]. On the other hand, Saneto et al. [12] reported that radical therapy for elderly HCC patients with HCV infection improved their survival rate. However, the impact of virological factors on the clinical features and survival

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Suguru Ogura, MD
Department of Hepatology, Toranomon Hospital
2-2-2 Toranomon, Minato-ku, Tokyo 105-0001 (Japan)
Tel. +81 44 877 5111, Fax +81 44 860 1623
E-Mail suguru-o@toranomon.gr.jp

rate after treatment of elderly patients with HCC is not clear at present.

Previous studies indicated that amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of genotype 1b are predictors of poor virological response to peginterferon + ribavirin therapy [14–20], and also risk factors for hepatocarcinogenesis [21, 22]. However, it is not clear at this stage whether aa substitutions in the core region of genotype 1b influence the clinical features and survival rate after treatment of HCC patients.

The study subjects were 136 consecutive patients infected with HCV, including 96 patients infected with HCV-1b. The aims of the study were the following: (1) to compare the clinicopathological features and virological factors of elderly patients (≥75 years old) with HCV infection, with those of young patients (≤50 years old), with a special emphasis on those with HCV-1b, which is the dominant genotype in Japan, and (2) to analyze the predictive factors, especially virological factors, associated with mortality in HCV-1b patients who received first therapy for HCC.

Methods

Patients

From January 1980 to December 2005, 1,804 HCC patients were treated at Toranomon Hospital, Tokyo, Japan. HCV antibody-positive and hepatitis B surface antigen-negative patients were included in this study. Of these consecutive patients, 92 (5.1%) were ≥75 years old, while 44 (2.4%) were ≤50 at the time of development of primary HCC. We defined those aged ≥75 years as the 'elderly' group and those aged ≤50 as the 'young' group. The HCV genotype 1b-related HCC group (n = 96) comprised 62 elderly patients and 34 young patients. The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

We reviewed the medical records of the patients and compared their clinical characteristics and laboratory data. Sex, HCV genotype, history of IFN therapy, history of blood transfusion, alcohol consumption, presence of diabetes mellitus (only patients who needed treatment with oral antidiabetic agents or insulin injections), body weight, body mass index (BMI), family history of liver disease (i.e., chronic hepatitis, liver cirrhosis or HCC, whether or not with HCV infection) were recorded. In this study, diabetes mellitus was diagnosed based on treatment of diabetes rather than by analysis of HbA1c data.

To distinguish chronic hepatitis from cirrhosis, we used the discriminate score reported previously by our institution [23]. In brief, the score was generated by stepwise selection of 20 variables from among 168 peritoneoscopy- and biopsy-proven patients with chronic hepatitis and 37 patients with cirrhosis who were infected with HCV. It is calculated as follows: z = [0.124 × γ-globulin (%)] + [0.001 × hyaluronic acid (μg/l)] – [0.075 × platelet count (×10^4/μl)] – [0.413 × sex (male, 1; female, 2)] – 2.005. A positive z value denotes cirrhosis and a negative value indicates chronic hepatitis.

Laboratory Data

For laboratory data, we recorded platelet count, prothrombin activity, total bilirubin (T-Bil), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, α-fetoprotein (AFP), the retention rate of indocyanine green dye at 15 min (ICG R15), hyaluronic acid, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting glucose (FBG), serum insulin, serum iron, ferritin and levels of HCV RNA (TaqMan HCV PCR [24]).

Nucleotide Sequencing of HCV-1b Core and NS5A Gene

With the use of HCV-J (accession No. D90208) as a reference [25], the sequence of 1–191 aa in the core protein of genotype 1b was determined and then compared with the consensus sequence constructed from 50 clinical samples to detect substitutions at aa70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa91 of leucine (Leu91) or methionine (Met91) [14]. The sequence of 2209–2248 aa in the NS5A of genotype 1b (IFN-sensitivity determining region [ISDR]) reported by Enomoto et al. [26, 27] was also determined, and the numbers of aa substitutions in ISDR were defined as wild-type (≤1) or mutant type (≥2).

The aa substitutions of the core region and NS5A-ISDR were analyzed by direct sequencing [14, 26, 27]. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo). Nucleic acids were amplified by PCR using the following primers: (a) Nucleotide sequences of the core region: the first-round PCR was performed with CC11 (sense, 5′-GCC ATA GTG GTC TGC GGA AC-3′) and e14 (antisense, 5′-GGA GCA GTC CTT CGT GAC ATG-3′) primers, and the second-round PCR with CC9 (sense, 5′-GCT AGC CGA GTA GTG TT-3′) and e14 (antisense) primers. (b) Nucleotide sequences of NS5A-ISDR: the first-round PCR was performed with ISDR1 (sense, 5′-ATG CCC ATG CCA GGT TCC AG-3′) and ISDR2 (antisense, 5′-AGC TCC GCC AAG GCA GAA GA-3′) primers, and the second-round PCR with ISDR3 (sense, 5′-ACC GGA TGT GGC AGT GCT CA-3′) and ISDR4 (antisense, 5′-GTA ATC CGG GCG TGC CCA TA-3′) primers ([a] hemi-nested PCR; [b] nested PCR). All samples were initially denatured at 95° for 15 min. The 35 cycles of amplification were set as follows: denaturation for 1 min at 94°, annealing of primers for 2 min at 55°, and extension for 3 min at 72° with an additional 7 min for extension. Then 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo).

Hepatocellular Carcinoma

The diagnosis of HCC was based on imaging studies and histopathological examination. However, when a typical hypervascular staining pattern was obtained on angiography or a hyperattenuating nodule was detected on the arterial phase of dynamic

computed tomography, the nodule was considered HCC without histopathological examination. On the other hand, when the hepatic nodule did not show the aforementioned patterns, a fine-needle biopsy was performed to exclude or diagnose HCC. Tumor numbers, size, tumor marker levels (AFP), the first therapy method for HCC, recurrence of HCC after the first therapy, and the survival rate were also recorded.

Statistical Analysis

Non-parametric tests (χ^2 test and Mann-Whitney U-test) were used to compare the characteristics of these groups. The cumulative survival rate was calculated using the Kaplan-Meier technique, differences between the survival curves were tested using the log-rank test. Statistical analyses of survival according to specific variables were calculated period from the first treatment for HCC. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with mortality. We also calculated the odds ratios and 95% confidence intervals (95% CI). The potential factors associated with mortality included the following variables: sex, family history of liver disease, history of blood transfusion, history of IFN therapy, alcohol consumption (total amount of ethanol consumption by the time of HCC diagnosis, ≥500 kg), diabetes mellitus under treatment, body weight, BMI, prothrombin activity (PT), platelet counts, serum albumin, total bilirubin (T-Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), ICG R15, TC, HDL-C, (LDL-C, TG, serum iron, ferritin, fasting FBG, insulin, HOMA-IR, levels of HCV RNA, AFP, aa substitution in the core and ISDR of NS5A. We considered variables with $p < 0.05$ as significant and those with $p < 0.10$ as marginally significant. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, Ill., USA).

Results

Patient Characteristics with HCV Infection

Table 1 shows the clinical characteristics and laboratory data of the young and elderly groups with HCV-related HCC. Among the 92 elderly patients, 53% were men and 47% were women. For the younger group, 89% were men and 11% were women. The proportion of female patients was significantly higher in the elderly group than in the young group ($p < 0.001$). The proportion of patients with HCV genotype 1b was not different between two age groups ($p = 0.366$). Patients with family history of liver disease were more common in the young group than the elderly group (30.4 vs. 5.4%, $p < 0.001$). A larger proportion of elderly patients had history of blood transfusion than the younger group (32.6 vs. 52.2%, $p < 0.001$). On the other hand, a larger proportion of younger patients were alcohol drinkers than in the elderly group (23.9 vs. 10.9%, $p = 0.044$). Most of the elderly patients had not been treated with IFN before the diagnosis of HCC (37.0 vs. 6.5%, $p < 0.001$). Body weight and BMI were lower in the elderly group than in the younger group ($p < 0.001$ in both variables). In the elderly group, PT was higher than in the younger group ($p < 0.001$), whereas T-Bil and ALT were lower in the elderly group ($p < 0.001$ and $p = 0.035$, respectively). Serum insulin level and HOMA-IR were lower in the elderly group than in the younger group ($p = 0.029$ and $p = 0.032$, respectively). There were no significant differences between two groups in other variables.

Patient Characteristics with HCV-1b Infection

Table 2 shows the clinical characteristics and laboratory data of the young and elderly HCC groups with HCV-1b infection. Men formed 53% and women 47% of the 62 elderly patients, while in the younger group, these were 91 and 9%, respectively. The proportion of female patients was significantly higher in the elderly group than in the young group ($p < 0.001$). Patients with family history of liver disease were more common in the young than elderly group (29.4 vs. 5.4%, $p < 0.001$). More elderly patients had a history of blood transfusion than the younger group (51.6 vs. 26.5%, $p < 0.016$). However, the proportion of alcohol drinkers was higher for the younger group than elderly group (20.6 vs. 4.8%, $p = 0.029$). A higher proportion of elderly patients had not been treated with IFN before the diagnosis of HCC (41.2 vs. 8.1%, $p < 0.001$). Body weight and BMI were lower in the elderly group than in the younger group ($p < 0.001$ in both variables). In the elderly group, PT was higher than in the younger group ($p = 0.001$), whereas T-Bil and ALT was lower in the elderly group ($p < 0.001$ and $p = 0.006$, respectively). Serum insulin level and HOMA-IR were lower in the elderly than in the younger group ($p = 0.034$ and $p = 0.023$, respectively). HDL-C level was higher in the HCV-1b infection-HCC elderly group than the younger group. There were no significant differences in other factors between the two groups.

Survival According to aa Substitutions of HCV-1b Core Region

We analyzed the survival rates after the first treatment for HCC, according to substitutions in core aa70 and aa91, using the Kaplan-Meier technique for elderly and young patients with HCV-1b infection-HCC. In the elderly patients, the cumulative survival rate according to substitutions of core aa70 was not different (log-rank test; $p = 0.821$, fig. 1a). For Arg70 and Gln70/His70, the cumulative survival rates were 46, 43% at the end of 3 years; 38, and 17% at the end of 5 years, respectively. However, for the core aa91, the cumulative survival rates varied significantly according to the type of substitution (log-rank test; $p = 0.009$, fig. 1b). For Leu91 and Met91, the cumula-

Table 1. Profile of elderly and young HCC patients with HCV infection

Variable	Young group (n = 46)	Elderly group (n = 92)	p value
Male/female	41/5	49/43	<0.001
Family history of liver disease	30.4% (14/46)	5.4% (5/92)	<0.001
History of blood transfusion	32.6% (15/46)	52.2% (48/92)	0.026
History of interferon therapy	37.0% (17/46)	6.5% (6/92)	<0.001
Alcohol consumption (>500 kg)	23.9% (11/46)	10.9% (10/92)	0.044
Diabetes mellitus (under treatment)	17.4% (8/46)	6.3% (13/92)	0.615
Discriminate score (>0/≤0/ND)	27/12/7	43/42/7	0.077
Body weight, kg[a]	68.5 (51.8–87.2)	51.7 (35.5–83.8)	<0.001
Body mass index[a]	23.8 (19.1–31.3)	22.2 (14.8–29.1)	<0.001
Prothrombin activity, %[a]	75 (13.4–100)	88.8 (49.9–125.4)	<0.001
Platelet count, $\times 10^4/\mu l$[a]	10.6 (2.8–25.7)	11.3 (3.2–31.7)	0.173
Serum albumin, g/dl[a]	3.5 (2.1–4.3)	3.5 (2.3–4.5)	0.776
T-Bil, mg/dl[a]	1.2 (0.4–6.4)	0.9 (0.5–2.9)	<0.001
AST, IU/l[a]	61 (22–311)	57 (20–138)	0.683
ALT, IU/l[a]	55 (10–425)	46 (10–162)	0.035
ICG R15, %[a]	32 (6–68)	30 (8–86)	0.458
Total cholesterol, mg/dl[a]	153 (85–207)	158 (91–233)	0.209
HDL-C, mg/dl[a]	41 (16–68)	42 (20–89)	0.137
LDL-C, mg/dl[a]	80 (38–141)	94 (52–165)	0.123
Triglyceride, mg/dl[a]	87 (38–538)	81 (33–254)	0.248
Serum iron, μg/dl[a]	169 (7–326)	135 (13–513)	0.169
Ferritin, μg/l[a]	325 (10–1,271)	89 (10–726)	0.063
Fasting blood glucose, mg/dl[a]	97 (39–313)	95 (68–159)	0.970
Insulin, μU/ml[a]	12.9 (3.6–71.2)	11.2 (1.9–69.9)	0.029
HOMA-IR[a]	3.2 (0.7–29.2)	2.6 (0.3–37.2)	0.032
Level of HCV RNA, log IU/ml[a]	5.6 (3.5–7.6)	6.1 (3.1–7.3)	0.177
AFP, μg/l[a]	29 (2–2,700)	37 (2–16,300)	0.841
Number of HCC, solitary/multiple	27/19	58/34	0.709
Size of largest tumor, mm[a]	20 (80–170)	20 (7–72)	0.716

HCC = Hepatocellular carcinoma; T-Bil = total bilirubin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ICG R15 = retention rate of indocyanine green dye at 15 min; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; AFP = α-fetoprotein; HOMA-IR = homeostasis model assessment of insulin resistance.

[a] Median (range).

tive survival rates were 57, 18% at the end of 3 years, and 41, 0% at the end of 5 years, respectively.

In the young patients, the cumulative survival rates were significantly different according to the type of substitution of core aa70 (log-rank test; p = 0.012, fig. 2a). For Arg70 and Gln70/His70, the cumulative survival rates were 82, 65% at the end of 3 years, and 60, 22% at the end of 5 years, respectively. For the core aa91, the cumulative survival rates according to the type of substitution were significantly different (log-rank test; p = 0.026, fig. 2b). For Leu91 and Met91, the cumulative survival rates were 87, 54% at the end of 3 years, and 60, 22% at the end of 5 years, respectively.

Multivariate Analysis of Factors Associated with Mortality after First Treatment of HCC

We then analyzed the data to determine those variables that influenced mortality after first treatment of HCC. Univariate analysis showed a relationship between each of the following six parameters and mortality in the elderly group: sex (male, p = 0.0287), treatment (non-radical therapy, p = 0.0203), PT (<80%, p = 0.0099), tumor size (≥30 mm, p = 0.0477), substitution of core aa91 (Met91, p = 0.0091) and serum iron level (≥121 μg/dl, p = 0.0526). These factors were entered into multivariate analysis, which then identified three independent parameters that tended to or significantly influenced mor-

Table 2. Profile of elderly and young HCC patients with HCV genotype 1b infection

	Young group (n = 34)	Elderly group (n = 62)	p value
Male/female	31/3	33/29	<0.001
Family history of liver disease	29.4% (10/34)	5.4% (3/62)	<0.001
History of blood transfusion	26.5% (9/34)	51.6% (32/62)	0.016
History of interferon therapy	41.2% (14/34)	8.1% (5/62)	<0.001
Alcohol consumption (≥500 kg)	20.6% (7/34)	4.8% (3/62)	0.029
Diabetes mellitus (under treatment)	20.6% (7/34)	14.5% (9/62)	0.400
Body weight, kg[a]	68.9 (51.8–87.2)	51.2 (35.5–83.8)	<0.001
Body mass index[a]	24.3 (19.1–31.3)	22.2 (14.8–29.1)	<0.001
Prothrombin activity, %[a]	78.8 (13.4–100)	90.1 (52.0–125.4)	0.001
Platelet count, $\times 10^4/\mu l$[a]	10.9 (2.8–25.7)	11.3 (3.2–31.7)	0.117
Serum albumin, g/dl[a]	3.4 (2.1–4.3)	3.5 (2.3–4.5)	0.199
T-Bil, mg/dl[a]	1.2 (0.4–6.4)	0.9 (0.5–2.9)	<0.001
AST, IU/l[a]	65 (22–229)	58 (20–138)	0.210
ALT, IU/l[a]	65 (10–295)	48 (11–106)	0.006
ICG R15, %[a]	32 (6–65)	32 (12–86)	0.410
Total cholesterol, mg/dl[a]	153 (85–197)	158 (91–233)	0.174
HDL-C, mg/dl[a]	38 (3–68)	44 (20–89)	0.010
LDL-C, mg/dl[a]	86 (41–122)	94 (52–165)	0.185
Triglyceride, mg/dl[a]	93 (38–538)	79 (33–223)	0.243
Serum iron, μg/dl[a]	123 (7–326)	138 (13–405)	0.655
Ferritin, μg/l[a]	140 (10–1,271)	88 (10–726)	0.323
Fasting blood glucose, mg/dl[a]	96 (56–313)	95 (68–159)	0.741
Insulin, μU/ml[a]	13.7 (3.6–204.0)	10.6 (1.9–102.0)	0.034
HOMA-IR[a]	3.7 (0.7–85.7)	2.6 (0.3–19.4)	0.023
Substitution of core amino acid 70 (Arg/Gln or His/ND)	17/11/6	24/18/20	0.809
Substitution of core amino acid 91 (Leu/Met/ND)	13/15/6	27/14/20	0.457
Mutation of NA5A-ISDR (≤1/>2/ND)	21/6/7	16/24/22	0.186
Level of HCV RNA, log IU/ml[a]	5.9 (3.5–7.6)	6.3 (3.4–7.3)	0.220
AFP, μg/l[a]	25 (2–1,690)	34 (2–1,150)	0.806
Number of HCC, solitary/multiple	14/20	21/41	0.293
Size of largest tumor, mm[a]	18 (8–170)	20 (7–72)	0.502

HCC = Hepatocellular carcinoma; T-Bil = total bilirubin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ICG R15 = retention rate of indocyanine green dye at 15 min; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; AFP = α-fetoprotein; HOMA-IR = homeostasis model assessment of insulin resistance; Arg = arginine; Gln = glutamine; His = histidine; Leu = leucine; Met = methionine.

[a] Median (range).

tality: sex (male, p = 0.021), substitution of core aa91 (Met91, p = 0.012), and treatment (non-radical therapy, p = 0.085) (table 3).

Similar analyses were conducted in the young group. Univariate analysis showed a relationship between each of the following parameters and mortality in the young group: first therapy (non-radical therapy, p = 0.0113), AFP (≥21 μg/l, p = 0.0329), AST (<57 IU/l, p = 0.0813), ALT (<51 IU/l, p = 0.0275), tumor number (solitary, p = 0.0048), presence of diabetes mellitus (p = 0.0001), FBG (≥111 mg/dl, p = 0.0217) and substitution of aa in core region 70 and 91 (Gln70/His70 and Met91, p = 0.0122 and p = 0.0258, respectively). These factors were entered into multivariate analysis, which then identified six independent parameters that tended to or significantly influenced mortality: treatment (non-radical therapy, p = 0.002), ALT (<51 IU/l, p = 0.045), AFP (≥21 μg/l, p = 0.021), FBG (≥111 mg/dl, p = 0.019), substitution of core aa91 (methionine, p = 0.011) and tumor number (solitary, p = 0.084) (table 4).

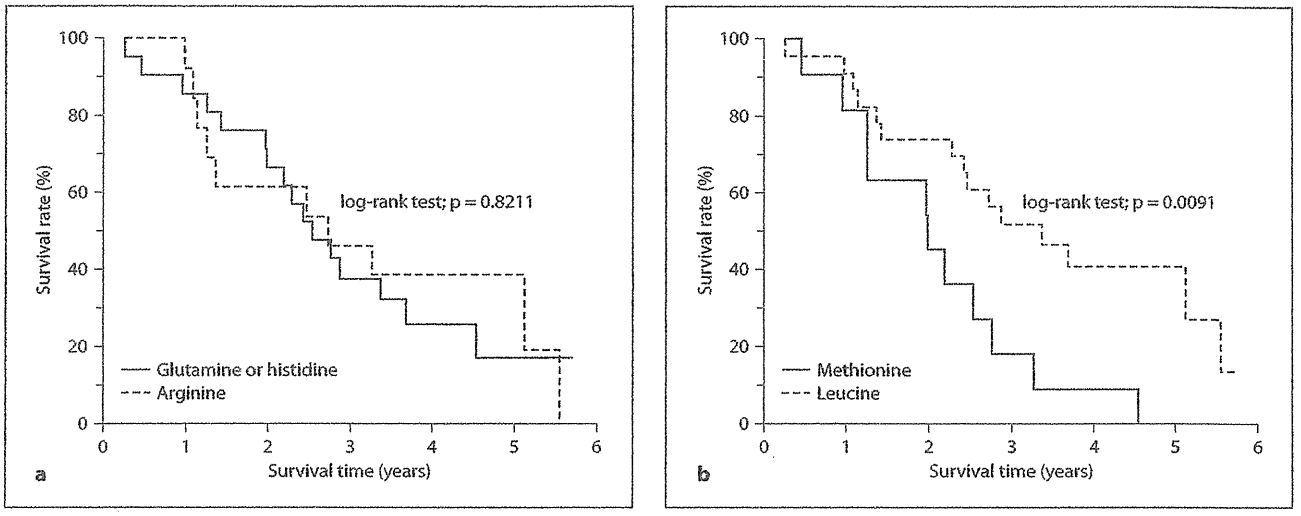

Fig. 1. Cumulative survival rates according to substitution of core amino acid in the elderly group. **a** Cumulative survival rate according to Gln70/His70 was not different compared with that related to Arg70. **b** Cumulative survival rate in Met91 was significantly lower than in Leu91.

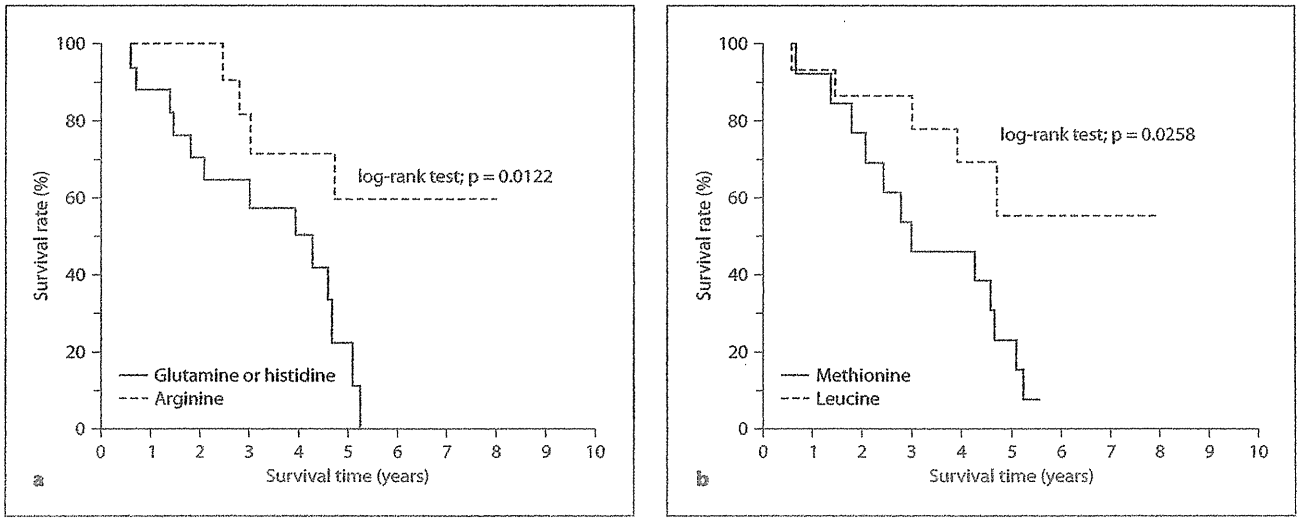

Fig. 2. Cumulative survival rates according to substitution of core amino acid in the young group. **a** The cumulative survival rate according to Gln70/His70 was significantly lower than that related to Arg70. **b** The cumulative survival rate according to Met91 was significantly lower than that related to Leu91.

Discussion

Previous studies reported that elderly patients could develop HCC even when liver histology shows chronic hepatitis and lack of cirrhosis and these patients included more females, compared with young patients [12, 13]. The present study compared the clinicopathological features of elderly and young patients with HCC based on HCV infection. Glucose metabolism, lipid metabolism, and liver function were preserved regardless of the stage of fi-

Table 3. Factors associated with mortality in elderly patients with HCV genotype 1b infection using multivariate analysis

Factors	Category	Odds ratio (95% CI)	p value
Sex	1: Female	1	
	2: Male	3.331 (1.184–7.717)	0.021
Core amino acid 91	1: Leucine	1	
	2: Methionine	3.393 (1.311–8.782)	0.012
First therapy for hepatocellular carcinoma	1: Radical therapy	1	
	2: Non-radical therapy	2.500 (0.881–7.098)	0.085

Radical therapy includes operation, percutaneous ethanol injection, percutaneous microwave coagulation therapy, and radiofrequency ablation.

Non-radical therapy includes transcatheter artery embolization and conservative therapy.

Only variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) in a multivariate Cox proportional hazard model are shown.

Table 4. Factors associated with mortality in young patients with HCV genotype 1b infection using multivariate analysis

Factors	Category	Odds ratio (95% CI)	p value
First therapy	1: Radical therapy	1	
	2: Non-radical therapy	22.04 (3.175–153.0)	0.002
AFP, μg/l	1: <21	1	
	2: ≥21	4.507 (1.251–16.23)	0.021
ALT, IU/l	1: ≥51	1	
	2: <51	3.401 (1.031–11.24)	0.045
Fasting blood glucose, mg/dl	1:<111	1	
	2:≥111	4.857 (1.295–18.21)	0.019
Core amino acid 91	1: Leucine	1	
	2: Methionine	6.091 (1.516–24.47)	0.011
Tumor number	1: Multiple	1	
	2: Solitary	3.546 (0.845–14.95)	0.084

Radical therapy includes operation, percutaneous ethanol injection, percutaneous microwave coagulation therapy, and radiofrequency ablation.

Non-radical therapy includes transcatheter artery embolization and conservative therapy.

AFP = α-Fetoprotein; ALT = alanine aminotransferase.

Only variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) in a multivariate Cox proportional hazard model are shown.

brosis in elderly patients. A previous study reported that liver histology was a significant variable between elderly and young groups [12], but in the present study, liver histology was not a significant variable. The discrepancy between our result and that of the previous study may be due to two reasons. One reason may be the larger number of patients in this study than the previous study. The other reason may be that preserved liver function, glucose and lipid metabolism suggested better liver histology in our study. To our knowledge, the present study is the first to report preservation of glucose and lipid metabolism in elderly HCC patients. Further studies of larger number of patients are required to determine more distinct differences between the two groups.

The viral factors associated with hepatocarcinogenesis in patients infected with HCV are still incompletely investigated, though some have been clarified in recent studies. Ogata et al. [28] reported that HCV genotype 1b strains might be associated with HCC based on the secondary structures of the amino-terminal portion of the HCV NS3 protein. Giménez-Barcons et al. [29] reported that high aa variability within the NS5A of HCV might be associated with HCC in patients with HCV-1b-related cirrhosis. Our previous study also reported that aa substitutions of the HCV-1b core region were important predictor of hepatocarcinogenesis [21, 22]. Whereas the association of viral factor and hepatocarcinogenesis has been gradually clarified, the association of viral factor and mortality has not been investigated. In the present study, substitutions of Met91 in the core region were associated with mortality of elderly HCV-1b patients after first treatment for HCC. In young HCV-1b-HCC patients, substitutions of Met91 and/or Gln70/His70 were also associated with mortality. To our knowledge, our study is the first to report that aa substitutions of the HCV-1b core region could be related to mortality after first treatment of HCC in elderly and young patients. Admittedly, we did not investigate the clinical impact of other virological factors on survival, except for HCV core region and NS5A-ISDR. Therefore, further studies should be performed to investigate the clinical impact of other viral regions of HCV on survival.

Previous studies identified substitutions in aa70 and/or aa91 in the HCV-1b core region and elevated AFP levels as predictors of poor virological response to peginterferon + ribavirin therapy [14–20], and also as risk factors and surrogate markers of hepatocarcinogenesis [21, 22]. Our study is the first to report that aa substitutions of core region are associated with mortality of elderly and young patients with HCV-1b-HCC. Especially Met91 in the core region was a significant factor of mortality in elderly patients. The reason might be the higher recurrence rate after radical treatment for elderly Met91 patients with first developed HCC (data not shown). A previous study reported that substitution of aa70 in the core region was a more important predictor of elevated AFP as a surrogate marker of hepatocarcinogenesis than that of aa91 [22]. However, in this study, substitution of aa70 (Gln70/His70) in the core region was not a significant variable of mortality in the elderly group. This discrepancy might be explained by the higher levels of malignant potentiality of substitution in aa70. Accordingly, because patients with Gln70/His70 in the core region had already developed HCC at a younger age, this might tend to decrease the number of patients with Gln70/His70 in the elderly group and increase the number of patients with Met91 in the elderly group.

A previous study of low-dose intermittent IFN monotherapy showed that ALT normalization rates did not differ according to core aa substitutions, which is a poor response to peginterferon + ribavirin combination therapy and risk factor of hepatocarcinogenesis [30]. Thus, a low-dose intermittent IFN monotherapy for patients with aa substitutions (Met91 and/or Gln70/His70) is an efficacious therapeutic regimen for normalization of ALT and thus reduction of risk of hepatocarcinogenesis, and probably improvement of survival. Especially since female patients with Met91 and/or Gln70/His70 are a high-risk group of hepatocarcinogenesis when they age, such a group should receive low-dose intermittent IFN monotherapy to reduce the risk of hepatocarcinogenesis.

Previous studies examined patients with HCV and identified age, the extent of liver fibrosis, male sex, alcohol consumption, positivity for hepatitis B surface antigen, and high levels of AFP and γ-GTP as risk factors for hepatocarcinogenesis [31–38]. Furthermore, radical therapy improved survival of elderly HCV patients after first development of HCC [12]. In the present study, multivariate analysis in elderly patients with HCV-1b-related HCC identified viral factor (Met91), host factor (male sex), and therapeutic factor (non-radical therapy) as factors of mortality after treatment for HCC. Furthermore, in their young counterparts, multivariate analysis also identified viral factor (Met91), host factors (low levels of ALT and high levels of FBG), therapeutic factor (non-radical therapy), and tumor factors (high levels of AFP and solitary HCC) as factors of mortality. To our knowledge, this study is the first to report that viral factors are associated with mortality of HCV patients after first treatment for HCC. Further studies should be performed to investigate the association between virological factors and mortality of HCV patients.

The limitations of the present study were that it did not investigate dominant group of HCC (i.e., from 51 to 74 years of age), basic mechanism of association between aa substitutions of the core region and mortality, other genotypes apart from HCV-1b, the geographic diversities of HCV-1b core region, and the study of other races apart from Asians in Japan. Further prospective studies, matched for HCV genotype, aa substitutions of the core region, and race of a large group of patients are required to determine the association between viral factors and mortality.

In conclusion, the results of the present study compared the characteristics of elderly and young patients with HCV-related HCC and found that aa substitutions of the HCV-1b core region might be related to mortality of patients after first treatment of HCC. This finding highlights the importance of low-dose intermittent IFN monotherapy in reducing the risk of hepatocarcinogenesis in this group of patients.

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Original Article

Rapid loss of hepatitis C virus genotype 1b from serum in patients receiving a triple treatment with telaprevir (MP-424), pegylated interferon and ribavirin for 12 weeks

Fumitaka Suzuki,[1] Norio Akuta,[1] Yoshiyuki Suzuki,[1] Hitomi Sezaki,[1] Hiromi Yatsuji,[1] Yusuke Kawamura,[1] Tetsuya Hosaka,[1] Masahiro Kobayashi,[1] Yasuji Arase,[1] Kenji Ikeda,[1] Rie Mineta,[2] Satomi Iwasaki,[2] Sachiyo Watahiki,[2] Mariko Kobayashi,[2] Yuzo Miyakawa[3] and Hiromitsu Kumada[1]

[1]*Department of Hepatology and* [2]*Research Institute for Hepatology, Toranomon Hospital, Kawasaki City, and* [3]*Miyakawa Memorial Research Foundation, Tokyo, Japan*

Aim: To evaluate the efficacy and safety of the triple treatment with telaprevir (MP-424), pegylated interferon (PEG-IFN) and ribavirin during 12 weeks on-treatment.

Methods: The triple treatment was given to 20 patients with chronic hepatitis C who had been infected with hepatitis C virus (HCV)-1b in high viral load (median: 6.8 log IU/mL [range: 5.5–7.2]), with a median age of 54 years (range: 36–65 years). They were followed for early dynamics of HCV RNA in serum during 12 weeks and side-effects.

Results: HCV RNA levels decreased by 4.8 logs by 7 days and 5.5 logs by 14 days. HCV RNA disappeared in 50% (10/20) at 2 weeks, 79% (15/19) at 4 weeks, 88% (14/16) at 6 weeks, 94% (15/16) at 8 weeks and 100% (13/13) at 12 weeks. HCV RNA disappeared equally frequently in 10 treatment-naive patients, six non-responders to IFN monotherapy and four non-responders to PEG-IFN and ribavirin. It was no different in the patients with and without amino acid substitutions reducing the response to IFN. The treatment was withdrawn in seven (35%) patients, mostly due to reduced hemoglobin of less than 8.5 g/dL, of whom six (86%) remained clear of HCV RNA at 12 weeks.

Conclusion: HCV RNA was lost from serum rapidly and universally in patients infected with HCV-1b in high viral loads by the triple treatment. Because an early loss of HCV RNA correlates with high rates of sustained virological response (SVR), it would increase SVR substantially, and merit the patients who have not responded to previous therapies.

Key words: chronic hepatitis, hepatitis C virus, interferon, ribavirin, telaprevir

INTRODUCTION

WORLDWIDE, AN ESTIMATED 170 million people are infected with hepatitis C virus (HCV) persistently.[1] Decompensated cirrhosis and hepatocellular carcinoma (HCC) develop in approximately 30% of individuals infected with HCV, and result in a fatal outcome during the lifetime.[2,3] At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for terminating HCV infection. The response to IFN is influenced by virological factors, such as viral load and genotypes, as well as host factors including sex, age and ethnicity, and responses to previous treatments.[4–6] In Japan, genotype 1b in high viral loads (> 100 KIU/mL) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C.[7–10] Because HCV started to spread during the turmoil surrounding the World War II (1939–1945) in Japan,[11] patients with chronic hepatitis C in need of treatment are entering their 50s and 60s by now. These backgrounds call for efficient treatments of patients with chronic hepatitis C in Japan.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, the sustained virological response (SVR) lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients with high-titer HCV-1b.[12,13] Recently, a new strategy was introduced to the treatment of chronic hepatitis C by means of

Correspondence: Dr Fumitaka Suzuki, Department of Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan. Email: fumitakas@toranomon.gr.jp
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inhibiting protease in NS3/NS4 of the HCV polyprotein. Of them, telaprevir (VX-950) has been selected as a clinical candidate for treatment of chronic hepatitis C.[14] Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity.[15,16] Thus, HCV RNA disappears in 95–100% of the patients infected with HCV-1 during the triple therapy for 14–28 days.[17–19] The SVR, lasting for longer than 24 weeks from the end of triple treatment, was not achieved in all patients who had received the triple treatment, however.[19]

In the present study, the triple therapy was given for 84 days to 20 Japanese patients infected with HCV-1b in high viral loads with the median age of 54 years (range: 36–65), including six non-responders to the standard IFN and four non-responders to PEG-IFN/ribavirin. They all lost HCV RNA from serum without major side-effects, providing a hope for better responses in the patients with chronic hepatitis C who have been refractory to previous IFN-based treatments.

METHODS

THIS DOUBLE-ARM, randomized, open-label study was conducted during April 2008 through March 2009 at the Department of Hepatology in the Toranomon Hospital at Metropolitan Tokyo in compliance with Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki. Before the study, the protocol and informed consent forms were approved by the institutional review board. All patients had given an informed consent in writing after sufficient explanation before they participated in this trial.

Patients

This study was performed on the 20 patients with chronic hepatitis C who met the following inclusion and exclusion criteria. Inclusion criteria for them were: (i) diagnosed with chronic hepatitis C; (ii) HCV-1b confirmed by the sequence analysis in the NS5B region; (iii) possessed HCV RNA levels of 5.0 log IU/mL or more determined with the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) Japanese (Mongoloid) aged 20–65 years at the entry; and (v) had a bodyweight of 35 kg or more and 120 kg or less at the time of registration.

Exclusion criteria were: (i) decompensated liver cirrhosis; (ii) hepatitis B surface antigen (HBsAg) in serum; (iii) HCC or its history; (iv) previous treatment for malignant neoplasm; (v) autoimmune hepatitis, alcohol liver disease, hemochromatosis or chronic liver disease other than chronic hepatitis C; (vi) depression, schizophrenia or suicide attempts; (vii) abnormal hemoglobin disease; (viii) angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, or their history; (ix) uncontrollable hypertension; (x) chronic renal dysfunction or creatinine clearance of 50 mL/min or less at baseline; (xi) hemoglobin level of 12 g/dL or less, neutrophil count of 1500/mm^3 or less or platelet count of 100 000/mm^3 or less at baseline; (xii) diabetes in need of treatment or fasting glucose level of 110 mg/dL or more at baseline; (xiii) autoimmune disease; (xiv) cerebrovascular disorder or its history; (xv) thyroidal dysfunction uncontrollable by medical treatment; (xvi) chronic pulmonary disease; (xvii) a history of allergy to medication or anaphylactoid symptoms; (xviii) women who were pregnant, breast-feeding or who could become pregnant; and (xix) men with a pregnant partner.

Study design

The 20 patients were randomly allocated to two groups with different doses of telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) by a third party institute, Bellsystem24 (Tokyo, Japan). MP-424 was administered in doses of 750 mg (group A) or 500 mg (group B) three times a day at an 8-h (q8) interval after the meal. PEG-IFN-α-2b (PEG-Intron; Schering Plough, Kenklworth, NJ, USA) was injected s.c. at a median dose of 1.5 μg/kg (range: 1.250–1.739 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at a dose of 200–600 mg twice a day after breakfast and dinner (daily dose: 600–1000 mg). These three drugs were administered for 12 weeks. After completion or discontinuation of the triple therapy, a follow-up observation was performed for 24 weeks.

Doses of PEG-IFN and ribavirin were reduced or their administration discontinued, as required, based on the reduction of hemoglobin levels, white blood cell count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced to one half, when leukocyte count decreased below 1500/mm^3, neutrophil count below 750/mm^3 or platelet count below 80×10^3/mm^3; PEG-IFN was withdrawn when they decreased below 1000/mm^3, 500/mm^3 or 50×10^3/mm^3, respectively. When hemoglobin decreased below 10 g/dL, the daily dose of ribavirin was reduced from 600 to 400 mg, from 800 to 600 mg and 1000 to 600 mg, depending on the initial dose of each patient. Ribavirin was withdrawn when hemoglobin decreased below 8.5 g/dL. The decrease of telaprevir (MP-424) dose was not permitted, and its administra-

tion was stopped when the discontinuation was appropriate for the development of adverse events. In cases when the administration of MP-424 was stopped, the administration of PEG-IFN-α-2b and ribavirin was terminated, also.

HCV RNA measurements

Antiviral effects of telaprevir (MP-424) on HCV were assessed by measuring plasma HCV RNA levels. Blood samples were obtained on day 1 before dosing and at 2.5, 4, 8, 16 and 24 h after the first dose (the 8-h sample was collected before administration of dose 2, and the 16-h sample was collected before administration of dose 3). Pre-dose samples were obtained on days 2, 3, 8, 14, 29, 43, 57, 86, 92, 99, 113, 141, 169, 197, 225 and 253. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/mL.

Safety assessments

During the on-study period, patients were monitored for safety at regular intervals from the start of dosing through every hospital visit. Safety assessments included physical examinations, clinical laboratory tests and adverse events. After the treatment was completed or aborted, patients were monitored for safety by the standard practice of investigators.

Statistical analysis

No prospective calculations of the statistical power were made. The sample size was selected to provide information on the safety and tolerability during 12 weeks of dosing. Descriptive statistics were reported for clinical laboratory and vital sign data. Categorical presentation was used for adverse events. HCV RNA values in log IU/mL were summarized using descriptive statistics for each treatment group and scheduled time point.

RESULTS

Baseline demographic and virological characteristics of the 20 patients with chronic hepatitis C who received the triple treatment

TABLE 1 LISTS baseline characteristics of the 20 patients who received the triple treatment with telaprevir (MP-424), PEG-IFN and ribavirin for 12 weeks. They all were infected with HCV-1b in high viral loads with a median of 6.8 log IU/mL. They were aged with a median of 54 years, and 14 (70%) of them were older than 50 years.

Table 1 Baseline characteristics of patients with chronic hepatitis C who received a triple therapy with telaprevir (MP-424), PEG-IFN and ribavirin

Male	10 (50%)
Age (years)	54 (36–65)
Hemoglobin (g/dL)	14.2 (12.1–16.8)
Platelets ($\times 10^3/mm^3$)	163 (102–243)
Albumin (g/dL)	4.2 (3.7–4.6)
Total bilirubin (mg/dL)	0.8 (0.3–1.1)
Creatinine (mg/dL)	0.76 (0.45–0.93)
Total cholesterol (mg/dL)	184 (114–253)
Fasting blood sugar (mg/dL)	112 (84–146)
Aspartate aminotransferase (IU/L)	40 (23–99)
γ-Glutamyl transpeptidase (IU/L)	39 (7–142)
Alanine aminotransferase (IU/L)	50 (26–167)
Hepatitis C virus RNA (log IU/mL)	6.8 (5.5–7.2)

Values are number with percentage in parentheses or median with range in parentheses.

Factors influencing the response to antiviral treatments are listed in Table 2. Of the 20 patients, 10 (50%) had not received antiviral treatments before, six (30%) had not responded to previous monotherapy with the standard IFN, and four (20%) had failed to respond to PEG-IFN and ribavirin before. Substitution of amino acid 70 in the HCV core protein from arginine to glutamine, as well as that of aa91 from leucine to methionine, interferes with the response to IFN-based treatments.[20,21] Such substitutions were detected in HCV RNA sequences from 10 (50%) patients. Amino acid substitutions in the IFN sensitivity determining region (ISDR) in the NS5A region, which increase the sensitivity to IFN,[22] were found in HCV RNA sequences from six (30%) patients.

Higher ages and poor responses to previous IFN-based treatments as well as high HCV-1b loads of the 20 patients, combined with virological characteristics diminishing the response in substantial subpopulations, forecast that they would likely be refractory to antiviral therapies.

Dynamics of HCV RNA in patients during the triple treatment

Figure 1 illustrates dynamics of HCV RNA in the 20 patients who had received the triple therapy with telaprevir (MP-424), PEG-IFN and ribavirin during 12 weeks. The effect of triple therapy in suppressing

Table 2 Amino acid substitutions interfering the response to IFN in patients who received telaprevir (MP-424)/PEG-IFN/ribavirin

Group and case no.	Age/Sex	Amino acids 70/91 in the core protein†	Amino acid substitutions in ISDR‡
(A) Treatment-naive patients ($n = 10$)			
1	36/F	Arg/Leu	1
2	47/F	Arg/Leu	0
3	47/F	**Glu**/Leu	1
4	54/F	Arg/Leu	1
5	60/F	Arg/Leu	0
6	62/F	Arg/Leu	1
7	44/M	Arg/**Met**	1
8	46/M	**Glu**/Leu	0
9	54/M	**Glu**/Leu	0
10	64/M	**Glu**/Leu	6
(B) Relapsers to previous IFN monotherapy ($n = 6$)			
11	54/F	Arg/Leu	0
12	56/F	Arg/**Met**	0
13	60/F	Arg/Leu	0
14	42/M	**Glu**/Leu	0
15	47/M	Arg/Leu	0
16	65/M	Arg/Leu	0
(C) Non-responders to combined PEG-IFN and ribavirin ($n = 4$)			
17	56/F	**Glu**/**Met**	0
18	47/M	Arg/Leu	0
19	52/M	**Glu**/**Met**	0
20	54/M	**Glu**/**Met**	0

†Substituted amino acids are marked in the boldface. ‡Numbers of amino acid substitutions in the interferon sensitivity determining region is shown. Arg, arginine; Glu, glutamine; IFN, interferon; ISDR, interferon sensitivity determining region; Leu, leucine; Met, methionine; PEG, pegylated.

HCV RNA levels was rapid, robust and universal. During the initial few days, HCV RNA dropped sharply (rapid phase) followed by slower decrease until 2 weeks (slow phase). The loss of detectable HCV RNA from serum (< 1.2 log IU/mL) occurred in 10 (50%) patients by 2 weeks. It gradually increased thereafter, and all of the 13 patients were freed of serum HCV RNA at the end of the 12-week therapy.

Viral dynamics during the triple therapy and previous PEG-IFN/ribavirin treatment in the four non-responders to combined treatment are illustrated in Figure 2. After the triple therapy, HCV RNA levels dropped sharply within 1 week in them all. The loss of HCV RNA from serum occurred at 2, 3, 4 and 8 weeks in them, respectively. In outstanding contrast, HCV RNA stayed in high

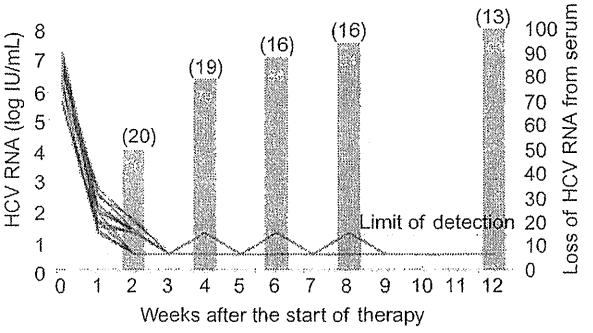

Figure 1 Dynamics of hepatitis C virus (HCV) RNA in the 20 patients with chronic hepatitis C during the triple treatment with telaprevir (MP-424), pegylated interferon (PEG-IFN) and ribavirin. The course of HCV RNA individual patients are indicated by lines, and loss of HCV RNA from serum by columns. The number of patients is indicated on the top of each column in parentheses, and areas below the sensitivity of detection are indicated by a shaded bar (< 1.2 log IU/mL).

levels during previous PEG-IFN and ribavirin treatment in all of them.

Early decreases in HCV RNA levels at 7 and 14 days during the triple treatment

Table 3 compares the average HCV RNA titers at baseline, 7 and 14 days during the triple treatment. Overall, HCV RNA decreased by 5.0 logs at 7 days and by 5.7 logs at 14 days. Similar decreases were achieved in subgroups of patients with various records of previous IFN-based treatment, and with or without amino-acid substitutions for a poor response to IFN. Early virological response was no different, either, between men and women (before: 6.63 ± 0.56 vs 6.50 ± 0.56; 1 week: 1.87 ± 0.57 vs 1.37 ± 0.41; and 2 weeks: 1.04 ± 0.44 vs 0.68 ± 0.30 log copies/mL, respectively), between the patients aged below and above 50 years of age, or between the patients who received two different doses of telaprevir (MP-424) (data not shown).

Normalization of alanine aminotransferase levels during the triple treatment

Normalization of alanine aminotransferase (ALT) levels in male and female patients during the triple treatment is summarized in Table 4. ALT levels were normalized (≤ 40 IU/L) in 80–90% of men at 2 weeks and thereafter. The normalization stayed below 60% in women due to dropouts increasing with the duration of triple treatment. Of the six women who had normalized ALT at 2 weeks, the triple treatment was terminated in one by

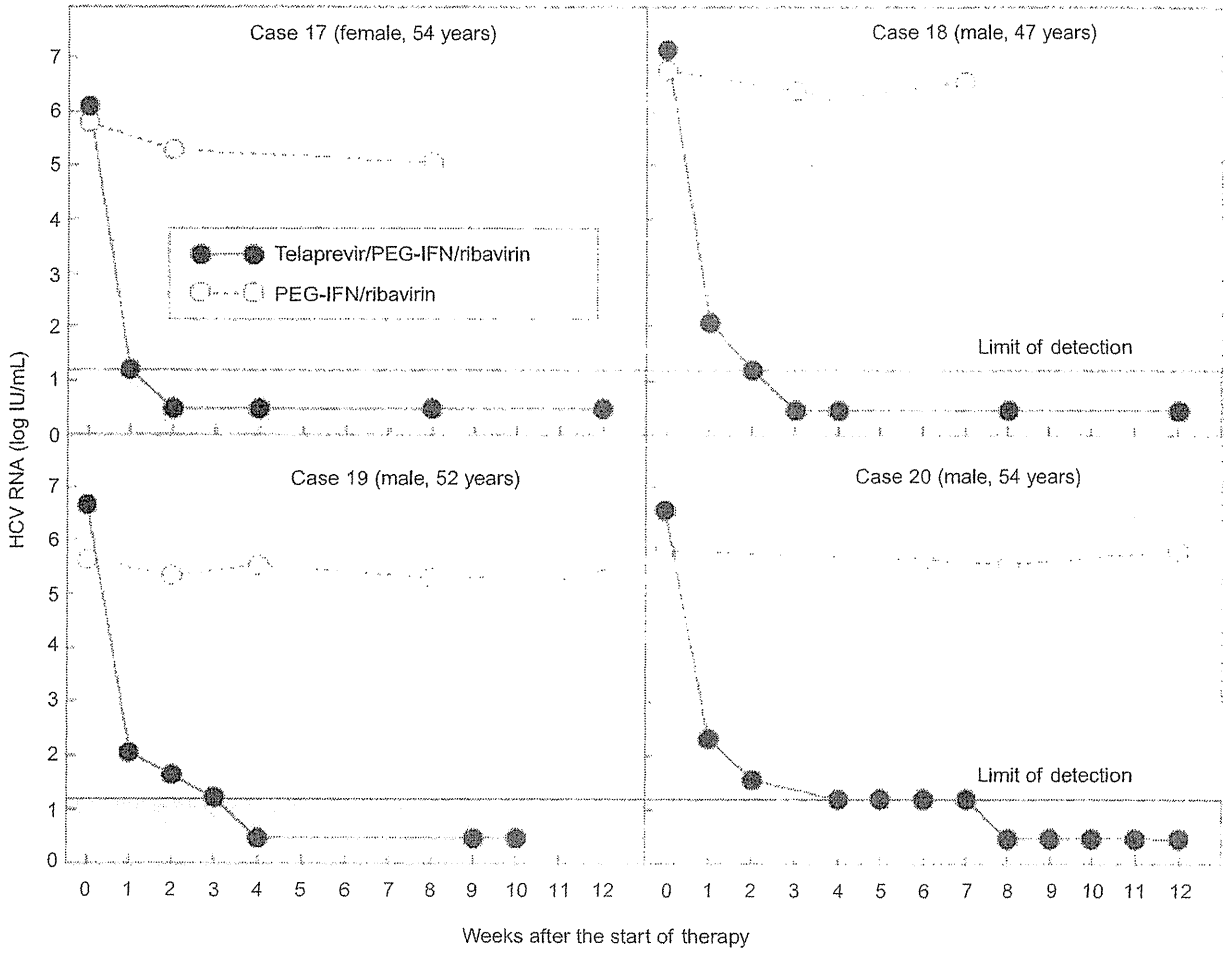

Figure 2 Dynamics of hepatitis C virus (HCV) RNA in non-responders to pegylated interferon (PEG-IFN) and ribavirin during the triple treatment. Black dots represent HCV RNA levels during the triple therapy, and open dots indicate HCV RNA levels during PEG-IFN and ribavirin they had received before.

4 weeks, four by 8 weeks and one by 12 weeks. Thus, the triple treatment was withdrawn in six of the 10 (60%) women, in remarkable contrast to only one of the 10 (10%) men. Overall, the normalization of ALT levels was achieved in 10 of the 13 (77%) patients who had completed the triple treatment for 12 weeks.

Side-effects during the triple treatment for 12 weeks

The triple treatment had to be stopped due to side-effects in seven of the 20 (35%) patients due to following reasons. Levels of hemoglobin during the triple treatment in each patient are illustrated in Figure 3. Five patients (one man and four women [cases 2, 13, 6, 1 and 9 in Table 2]) developed low hemoglobinemia (< 8.5 g/dL) at day 22, 29, 36, 78 and 84, respectively, after the start of triple treatment. One patient (female, 54 years [case 4]) came down with IFN-related symptoms including general malaise, and another (female, 56 years [case 12]) developed generalized dermatitis that was unable to be treated with topical steroid ointments.

DISCUSSION

IN JAPAN, THE majority of patients with chronic hepatitis C are infected with HCV-1b in high titers. Combined with their ages being approximately 20 years

Table 3 Early virological response to telaprevir (MP-424)/PEG-IFN/ribavirin in the patients with risk factors for non-response to therapy

Risk factors	*n*	HCV RNA (log IU/mL)†		
		Before	At 7 days	At 14 days
Previous interferon-based therapy				
Treatment-naive	10	6.4 ± 0.6	1.5 ± 0.5	0.8 ± 0.4
Non-responders to the standard IFN	6	6.8 ± 0.4	1.6 ± 0.6	0.9 ± 0.4
Non responders to PEG-IFN/ribavirin	4	6.7 ± 0.5	1.9 ± 0.6	1.1 ± 0.5
Amino acid substitutions in positions 70 and 91 in the core protein				
Arg/Leu	10	6.7 ± 0.5	1.6 ± 0.4	0.7 ± 0.3
Others	10	6.4 ± 0.6	1.7 ± 0.7	1.0 ± 0.5
Amino acid substitutions in the interferon sensitivity determining region (ISDR)				
0	14	6.8 ± 0.4	1.8 ± 0.6	1.0 ± 0.4
≥ 1	6	6.1 ± 0.6	1.3 ± 0.3	0.6 ± 0.2
Total	20	6.6 ± 0.5	1.6 ± 0.5	0.9 ± 0.4

†Values indicate the mean ± SD. Arg, arginine; HCV, hepatitis C virus; IFN, interferon; ISDR, interferon sensitivity determining region; Leu, leucine; PEG, pegylated.

older than those in Western countries,[11] the history of treatment response in Japanese patients with chronic hepatitis has been distressing. SVR was gained in merely 6% of patients with HCV-1b in high viral loads when natural IFN for 6 months was started in 1992; it increased to 20% with the standard IFN combined with ribavirin for 6 months implemented in 2001.[7–9] Finally, the introduction of PEG-IFN and ribavirin for 12 months approved in 2004 achieved SVR in 50% of them.[12,13] Another half of patients with chronic hepatitis C have been left with no means of eliminating HCV infection from them, which is utterly unacceptable.

Instigated by favorable results of short-term telaprevir in combination with PEG-IFN and ribavirin,[17–19] a Phase-I PK (pharmacokinetics) trial was started in the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. The results of the 20 patients with high-load HCV-1b infections, who had received the triple treatment for 12 weeks, are even more promising than those in previous reports with a treatment duration up to 4 weeks.[17–19]

Non-responders to IFN-based treatments, as well as relapsers among responders, are refractory to re-treatments with IFN.[4] The single salient result of the present study is that all the four non-responders to previous PEG-IFN and ribavirin behaved equally well as 10 treatment-naive patients and six non-responders to the standard IFN before. Virological responses to the triple treatment with telaprevir (MP-424), PEG-IFN and ribavirin was equally excellent, irrespective of amino acid substitutions in the core protein and ISDR that interfere with the effect of IFN-based treatments.[20–22] This is the best virtue of telaprevir (MP-424), and sends auspicious signals both to patients and doctors in charge of them. In fact, dynamics of HCV RNA during 12 weeks of the triple treatment was no different among treatment-naive patients, non-responders to previous treatment with the standard IFN or PEG-IFN and ribavirin (Fig. 1). The average HCV RNA titers of 6.4 log IU/mL at the baseline decreased by 5.0 logs at 7 days and by 5.7 logs at 14 days after the start of triple treatment (Table 3). Similar rapid and forceful decreases in HCV RNA levels have been reported unanimously in previous studies.[17–19]

Rapid, universal and robust antiviral activity of the triple therapy was not without costs. Anemia with

Table 4 Normalization of alanine aminotransferase levels during the triple treatment

	Before	2 weeks	4 weeks	8 weeks	12 weeks
Men	20% (2/10)	80% (8/10)	90% (9/10)	80% (8/10)	89% (8/9)
Women	20% (2/10)	60% (6/10)	56% (5/9)	33% (2/6)	50% (2/4)
Total	20% (4/20)	70% (14/20)	74% (14/19)	63% (10/16)	77% (10/13)

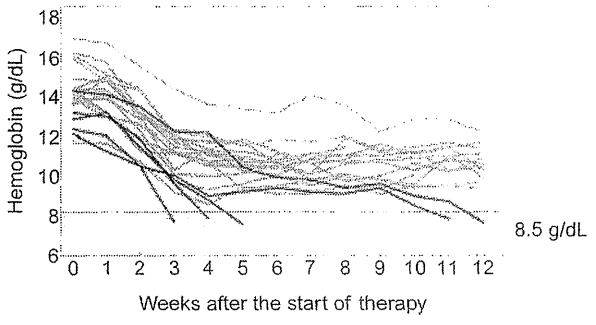

Figure 3 Levels of hemoglobin in the patients who received the triple treatment during 12 weeks. Black lines represent the patients in whom hemoglobin decreased below 8.5 g/dL and who were placed off the triple treatment at that point.

hemoglobin levels of less than 8.5 g/dL developed in five of them including one (10% [1/10]) man and four (40% [4/10]) women, and one each developed severe generalized dermatitis that could not be treated with topical steroids and general malaise that was ascribable to PEG-IFN. Thus, the triple treatment was withdrawn from seven of the 20 (35%) patients. Ribavirin dose would need to be monitored more closely and modified as soon as reduction is required. Such precaution would prevent aged women, who are especially prone to anemia, from discontinuing the triple treatment. Despite withdrawal of the triple treatment 22–89 days after the start, HCV RNA stayed below detectable levels (< 1.2 log IU/mL) in six (86%) of them at 12 weeks.

No virological breakthroughs with increases in HCV RNA levels occurred in any of the 20 patients during a 12-week triple therapy. Hence, HCV mutants with amino acid conversions for resistance to telaprevir, such as the 156S mutation,[23] would not have developed in them. Should they have elicited, they must have been suppressed by PEG-IFN given to the 20 patients under the triple therapy; telaprevir-resistant HCV mutants are susceptible to IFN in both *in vivo* and *in vitro* studies.[17,24]

Sustained virological response is the gold standard for evaluating the response to antiviral treatments. In large-scale follow-up studies, 99.2% of the patients who had achieved an SVR remained HCV RNA undetectable at a median of 4.1 years (range: 0.4–7.0).[25] There is a close correlation between the velocity of HCV RNA clearance and chances for SVR. Thus, SVR is achieved more often in the patients who did than who did not lose HCV RNA from serum within 12 weeks after the start of IFN-based treatment;[26] the faster HCV RNA is lost, the higher a chance for SVR.[21,27] In view of a rapid loss of HCV RNA from serum, the 20 patients studied will therefore have a great chance of achieving SVR, although this needs to be ascertained by follow ups longer than 24 weeks after the completion of triple therapy. In fact, SVR was not achieved in all patients who had received the triple treatment for 28 days.[19] It is a matter of conjecture how long the triple therapy should be continued and whether or not PEG-IFN and ribavirin need to be maintained after the withdrawal of telaprevir (MP-424). In any case, the duration of the triple treatment is reasonably expected to be shortened to less than that of PEG-IFN and ribavirin in the current use, towards ensuring less chances for telaprevir-resistant HCV variants to prevail and induce breakthrough hepatitis.

In conclusion, the triple treatment of the 20 patients with telaprevir (MP-424), PEG-IFN and ribavirin for 12 weeks suppressed HCV RNA in serum rapidly, universally and robustly, irrespective of previous responses to antiviral treatments or mutations decreasing the sensitivity to IFN. Promoted by few grave side-effects, the triple therapy is hoped to gain an excellent efficacy in treatment-resistant patients who are infected with HCV-1 in high viral loads.

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ORIGINAL ARTICLE

Development of hepatocellular carcinoma in elderly patients with chronic hepatitis C with or without elevated aspartate and alanine aminotransferase levels

MARIKO KOBAYASHI[1], FUMITAKA SUZUKI[2], NORIO AKUTA[2], YOSHIYUKI SUZUKI[2], HITOMI SEZAKI[2], HIROMI YATSUJI[2], YUSUKE KAWAMURA[2], TETSUYA HOSAKA[2], MASAHIRO KOBAYASHI[2], YASUJI ARASE[2], KENJI IKEDA[2], RIE MINETA[1], SATOMI IWASAKI[1], SACHIYO WATAHIKI[1], YUZO MIYAKAWA[3] & HIROMITSU KUMADA[2]

[1]*Research Institute for Hepatology,* [2]*Department of Hepatology, Toranomon Hospital, Tokyo, Japan, and* [3]*Miyakawa Memorial Research Foundation, Japan*

Abstract

Objective. Hepatocellular carcinoma (HCC) in the elderly infected with hepatitis C virus (HCV) is expected to increase globally within the next two decades. The purpose of the study was to define the natural history of elderly patients with chronic hepatitis C needs in order to prevent HCC from arising in these patients. ***Material and methods.*** Treatment-naive patients aged ≥65 years with platelet counts $>120\times10^3/mm^3$ were classified as 120 with aspartate and alanine aminotransferase (ASAT and ALAT) levels ≦40 IU/l (group A) and 212 with either or both levels ≥41 (group B) and followed-up for 3 years or longer without antiviral treatment. ***Results.*** Cirrhosis and HCC developed more frequently in group B than in group A ($p<0.001$ for both). In particular, of the patients aged 65–69 years at entry, cirrhosis and HCC developed more frequently in group B than in group A ($p<0.001$ and $p=0.001$, respectively). Liver-related causes of death were more common in group B than in group A (20/34 (59%) versus 1/9 (11%), $p=0.021$). HCC developed more frequently in men than in women ($p=0.033$). ***Conclusions.*** In elderly patients with chronic hepatitis C, cirrhosis and HCC develop more frequently in those with elevated transaminase levels than in those without elevated transaminase levels. Therefore, transaminase levels need to be suppressed below ≦40 IU/l, using antiviral treatments or other agents, in order to prevent cirrhosis and HCC arising in these patients. In view of rare liver-related deaths, aggressive antiviral treatment would not be necessary in the elderly with chronic hepatitis C who have normal transaminase levels.

Key Words: *Age, chronic hepatitis, cirrhosis, hepatitis C virus, hepatocellular carcinoma*

Introduction

There are an estimated 170 million people persistently infected with hepatitis C virus (HCV) worldwide, and approximately 30% of them develop serious complications during their lifetime, such as decompensated cirrhosis and hepatocellular carcinoma (HCC) [1]. The incidence of HCC in HCV carriers increases with age and is particularly high in those aged 65 years or older. Based on the shift in age-specific distribution of HCV carriers with time [2–4], HCC is expected to increase in the next 20 years, globally.

The natural history of infection with HCV is influenced by host and virological factors including age and gender [5–7], as well as viral loads and genotypes [8–10]. Thus, hepatitis proceeds slowly in HCV infections contracted by children and young women. During follow-ups carried out over 20 years, liver damage developed in a mere 3% of children who were infected with HCV during heart surgery [7], and cirrhosis emerged in only 2% of pregnant women infected with anti-D immune globulin contaminated with HCV [5].

As the average life span of human beings continues to extend, owing to improvements in sanitary

Correspondence: Mariko Kobayashi, BS, Research Institute for Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan. Tel: +81 44 8605 111. Fax: +81 44 8601 623. E-mail: vj7m-kbys@asahi-net.or.jp

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