

- 17 Laskus T, Rakela J, Tong MJ, Nowicki MJ, Mosley JW, Persing DH: Naturally occurring hepatitis B virus mutants with deletions in the core promoter region. *J Hepatol* 1994;20:837–841.
- 18 Laskus T, Rakela J, Nowicki MJ, Persing DH: Hepatitis B virus core promoter sequence analysis in fulminant and chronic hepatitis B. *Gastroenterology* 1995;109:1618–1623.
- 19 Takahashi K, Aoyama K, Ohno N, Iwata K, Akahane Y, Baba K, Yoshizawa H, Mishiro S: The precore/core promoter mutant (T1762/A1764) of hepatitis B virus: clinical significance and an easy method for detection. *J Gen Virol* 1995;76:3159–3164.
- 20 Lindh M, Horal P, Dhillon AP, Furuta Y, Norkrans G: Hepatitis B virus carriers without precore mutations in hepatitis B e antigen-negative stage show more severe liver damage. *Hepatology* 1996;24:494–501.
- 21 Erhardt A, Reineke U, Blondin D, Gerlich WH, Adams O, Heintges T, Niederau C, Häussinger D: Mutations of the core promoter and response to interferon treatment in chronic replicative hepatitis B. *Hepatology* 2000;31:716–725.
- 22 Usuda S, Okamoto H, Iwanari H, Baba K, Tsuda F, Miyakawa Y, Mayumi M: Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. *J Virol Methods* 1999;80:97–112.
- 23 Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M: Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* 1988;69:2575–2583.
- 24 Kwok S, Higuchi R: Avoiding false positive with PCR. *Nature* 1989;339:237–238.
- 25 Desmet VJ, Gerber M, Hoofnagle JH, Manna M, Scheuer PJ: Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994;19:1513–1520.
- 26 Lampertico P, Vigano M, Manenti E, Iavarone M, Sablon E, Colombo M: Low resistance to adefovir combined with lamivudine: a 3-year study of 145 lamivudine-resistant hepatitis B patients. *Gastroenterology* 2007;133:1445–1451.
- 27 Kobayashi M, Ikeda K, Hosaka T, Sezaki H, Someya T, Akuta N, Suzuki F, Suzuki Y, Saitoh S, Arase Y, Miyakawa Y, Kumada H: Natural history of compensated cirrhosis in the Child-Pugh class A compared between 490 patients with hepatitis C and 167 with B virus infections. *J Med Virol* 2006;78:459–465.
- 28 Bréchet C, Pourcel C, Louise A, Rain B, Tiollais P: Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. *Nature* 1980;286:533–535.
- 29 Bréchet C, Hadchouel M, Scotto J, Fonck M, Potet F, Vyas GN, Tiollais P: State of hepatitis B virus DNA in hepatocytes of patients with hepatitis B surface antigen-positive and -negative liver diseases. *Proc Natl Acad Sci USA* 1981;78:3906–3910.
- 30 Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC: Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. Studies in percutaneous liver biopsies and post-mortem tissue specimens. *N Engl J Med* 1981;305:1067–1073.
- 31 Wong DK, Yuen MF, Poon RT, Yuen JC, Fung J, Lai CL: Quantification of hepatitis B virus covalently closed circular DNA in patients with hepatocellular carcinoma. *J Hepatol* 2006;45:553–559.
- 32 Wursthorn K, Lugehetmann M, Dandri M, Volz T, Buggisch P, Zollner B, Longerich T, Schirmacher P, Metzler F, Zankel M, Fischer C, Currie G, Brosgart C, Petersen J: Peginterferon- α_{2b} plus adefovir induce strong cccDNA decline and HBsAg reduction in patients with chronic hepatitis B. *Hepatology* 2006;44:675–684.
- 33 Wong DK, Yuen MF, Ngai VW, Fung J, Lai CL: One-year entecavir and lamivudine therapy results in reduction of hepatitis B virus intrahepatic covalently closed circular DNA levels. *Antivir Ther* 2006;11:909–916.
- 34 Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF: Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999;29:971–975.
- 35 Lin SM, Yu ML, Lee CM, Chien RN, Sheen IS, Chu CM, Liaw YF: Interferon therapy in HBeAg-positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. *J Hepatol* 2007;46:45–52.

Amino Acid Substitutions in the Hepatitis C Virus Core Region of Genotype 1b Are the Important Predictor of Severe Insulin Resistance in Patients Without Cirrhosis and Diabetes Mellitus

Norio Akuta,^{1*} Fumitaka Suzuki,¹ Miharuru Hirakawa,¹ Yusuke Kawamura,¹ Hiromi Yatsuji,¹ Hitomi Sezaki,¹ Yoshiyuki Suzuki,¹ Tetsuya Hosaka,¹ Masahiro Kobayashi,¹ Mariko Kobayashi,² Satoshi Saitoh,¹ Yasuji Arase,¹ Kenji Ikeda,¹ and Hiromitsu Kumada¹

¹Department of Hepatology, Toranomon Hospital, Tokyo, Japan

²Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan

Previous studies provided a direct experimental evidence for the contribution of HCV core protein in the development of insulin resistance (IR), but the clinical impact of HCV core region on IR is still not clear. The present study evaluated the impact of Amino acid (aa) substitutions of HCV-1b core region on IR in 123 Japanese patients infected with HCV-1b without cirrhosis and diabetes mellitus, and investigated the treatment efficacy of 48-week pegylated interferon (PEG-IFN) plus ribavirin (RBV) according to HOMA-IR values. Patients with IR (HOMA-IR ≥ 2.5) and severe IR (HOMA-IR ≥ 3.5) were present in 51.2% and 27.6%, respectively. Multivariate analysis identified body mass index (≥ 25 kg/m²) and hepatocyte steatosis ($\geq 5\%$) as significant determinants of IR. Furthermore, multivariate analysis identified hepatocyte steatosis ($\geq 5\%$), aa substitutions of the core region (Gln70 (His70) and/or Met91), and age (≥ 55 years) as significant determinants of severe IR. Especially, significantly lower proportions of patients with Gln70 (His70) and/or Met91 were noted among those without severe IR (59.6%) than those with severe IR (82.4%). The rates of sustained virological response in patients with IR (50.0%) were not significantly different from those without IR (52.9%). Furthermore, the rates of non-virological response in patients with IR (28.9%) were not significantly also different from those without IR (20.6%). In conclusion, the present study indicated that substitutions of HCV-1b core region were the important predictor of severe IR in patients without cirrhosis and diabetes mellitus, but HOMA-IR values might be not useful as predictors of 48-week PEG-IFN plus RBV therapy. *J. Med. Virol.* 81:1032–1039, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: HCV; core region; genotype; HOMA-IR; hepatocyte steatosis; cirrhosis; diabetes mellitus

INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [Dusheiko, 1998; Ikeda et al., 1998; Niederau et al., 1998; Kenny-Walsh, 1999; Akuta et al., 2001]. Furthermore, HCV infection also affects an increased risk of diabetes mellitus [Allison et al., 1994; Caronia et al., 1999; Mason et al., 1999; Mehta et al., 2000, 2003; Zein et al., 2000, 2005; Antonelli et al., 2005] or insulin resistance (IR) [Hickman et al., 2003; Hui et al., 2003; Lecube et al., 2004, 2006]. IR and glucose metabolism impairment are associated with liver necroinflammation [Hui et al., 2003], hepatocyte steatosis [Fartoux et al., 2005; Cammà et al., 2006; Conjeevaram et al., 2007], cirrhosis [Petrides et al., 1994], and HCC [El-Serag et al., 2001; Lai et al., 2006; Veldt et al., 2008]. Especially, in patients infected with HCV genotype 1 (HCV-1), significant fibrosis is associated with IR independent from hepatocyte steatosis [Moucari et al., 2008; Petta et al., 2008].

Previous studies reported that HCV core protein induced HCC and IR in transgenic mice, and provided

Grant sponsor: Ministry of Health, Labor and Welfare, Japan (partly supported).

*Correspondence to: Norio Akuta, MD, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-0001, Japan. E-mail: akuta-gi@umin.ac.jp

Accepted 5 February 2009

DOI 10.1002/jmv.21473

Published online in Wiley InterScience (www.interscience.wiley.com)

a direct experimental evidence for the contribution of HCV core protein in the development of HCC and IR in human HCV infection [Moriya et al., 1998; Shintani et al., 2004]. Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of genotype 1b (HCV-1b core region) were predictors of poor virological response to 48-week pegylated interferon (PEG-IFN) plus ribavirin (RBV) combination therapy [Akuta et al., 2005, 2006, 2007a,b,c; Donlin et al., 2007; Okanoue et al., 2008], and also risk factors for hepatocarcinogenesis [Akuta et al., 2007d, 2008]. Thus, previous reports supported the oncogenic potential of the HCV core region and clinically linked substitutions of aa 70 and/or 91 in HCV-1b core region to HCC [Akuta et al., 2007d, 2008], but the clinical impact of HCV-1b core region on IR is still not clear. IR develops type 2 diabetes mellitus as its major late feature, and is also associated with advanced fibrosis [Petrides et al., 1994; Petta et al., 2008]. Hence, the biological mechanisms underlying the association between HCV core region and IR are probably multifactorial, and study based on patients without diabetes mellitus and cirrhosis, that might affect IR, should be performed to investigate whether HCV core region might affect IR clinically.

Previous reports showed that IR might be predictors of poor virological response to PEG-IFN plus RBV combination therapy [D'Souza et al., 2005; Romero-Gómez et al., 2005]. Chu et al. [2008] reported that IR was a major determinant of sustained virological response (SVR) in HCV-1 patients receiving 24-week PEG-IFN plus RBV. However, to our knowledge, there is little evidence that IR affects treatment efficacy of HCV-1b patients receiving 48-week PEG-IFN plus RBV combination therapy.

The aims of the present study conducted in Japanese patients infected with HCV-1b without cirrhosis and diabetes mellitus, were the following. (1) To evaluate the HOMA-IR values of patients infected with HCV-1b. (2) To identify the impact of aa substitutions in the core region on IR in such patients, and determine the factors associated with IR, and (3) to investigate the treatment efficacy of 48-week PEG-IFN plus RBV combination therapy according to HOMA-IR values.

PATIENTS AND METHODS

Study Population

At Toranomon Hospital, Tokyo, Japan, 221 HCV-infected Japanese patients were consecutively recruited into the study protocol of the combination therapy with PEG-IFN α -2b plus RBV between December of 2001 and June of 2005. Among these, 123 patients were selected in the present retrospective study based on the following criteria. (1) Negativity for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positivity for anti-HCV (third-generation enzyme immunoassay, Chiron Corp, Emerville, CA), and positivity for HCV RNA qualitative analysis with PCR (Amplicor, Roche Diagnostics, Mannheim, Germany). (2) They were infected with HCV-1b alone. (3) HOMA-IR values

and substitutions of aa 70 and 91 in the HCV core region were determined at the commencement of treatment. (4) They were free of cirrhosis and hepatocellular carcinoma, based on biopsy examination, laboratory tests, and imaging studies at baseline. (5) None had diabetes mellitus. (6) None was an alcoholic; lifetime cumulative alcohol intake was <500 kg (mild to moderate alcohol intake). (7) All were free of coinfection with human immunodeficiency virus. (8) None had other forms of hepatitis, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) Each signed a consent form of the study protocol that had been approved by the human ethics review committee. Table I summarizes the profiles and laboratory data of the 123 patients at the commencement of treatment. They included 71 males and 52 females, aged 20–70 years (median, 55 years). The treatment efficacy was evaluated by HCV-RNA positive based on qualitative PCR analysis at the end of treatment (non-virological response; NVR), and by HCV-RNA negative based on qualitative PCR analysis at 24 weeks after the completion of therapy (SVR).

Laboratory Tests

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for alanine aminotransferase (ALT) and HCV-RNA levels. The serum samples were frozen at -80°C within 4 hr of collection and thawed at the time of measurement. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of NS5 region [Chayama et al., 1993]. HCV-RNA levels were measured by quantitative PCR (AMPLICOR GT HCV Monitor v2.0 using the 10-fold dilution method, Roche Molecular Systems, Inc.) at least once every month before, during, and after therapy. The dynamic range of the assay was 5.0×10^3 to 5.0×10^6 IU/ml. Samples collected during and after therapy that showed undetectable levels of HCV-RNA ($<5.0 \times 10^3$ IU/ml) were also checked by qualitative PCR (AMPLICOR HCV v2.0, Roche Molecular Systems, Inc.), which has a higher sensitivity than quantitative analysis, and the results were expressed as positive or negative. The lower limit of the assay was 50 IU/ml.

Histopathological Examination of Liver Biopsies

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan). The biopsy material was fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. All specimens for examination contained six or more portal areas. Histopathological diagnosis was confirmed by an experienced liver pathologist (H.K.) who was blinded to the clinical data. Chronic hepatitis and liver cirrhosis were diagnosed

TABLE I. Profile and Laboratory Data of 123 Patients Infected With HCV Genotype 1b

Demographic data	
Number of patients	123
Sex (M/F)	71/52
Age (years)*	55 (20–70)
History of blood transfusion	41 (33.3%)
Family history of liver disease	37 (30.1%)
Body mass index (kg/m ²)*	23.6 (17.6–32.0)
Laboratory data*	
Serum aspartate aminotransferase (IU/L)	59 (17–266)
Serum alanine aminotransferase (IU/L)	81 (25–504)
Serum albumin (g/dl)	3.8 (3.1–4.5)
Gamma-glutamyl transpeptidase (IU/L)	50 (15–393)
Leukocytes (/mm ³)	4,800 (2,300–8,800)
Hemoglobin (g/dl)	14.4 (10.6–17.6)
Platelet count ($\times 10^4$ /mm ³)	16.8 (7.5–27.7)
Indocyanine green retention rate at 15 min (%)	15 (4–41)
Serum iron (μ g/dl)	138 (18–290)
Serum ferritin (μ g/L)	130 (<10–711)
Creatinine clearance (ml/min)	99 (46–146)
Level of viremia (KIU/ml)	1,900 (23–>5,000)
Alpha-fetoprotein (μ g/L)	6 (2–161)
Total cholesterol (mg/dl)	166 (96–294)
High-density lipoprotein cholesterol (mg/dl)	46 (10–83)
Low-density lipoprotein cholesterol (mg/dl)	101 (53–207)
Triglycerides (mg/dl)	95 (33–362)
Uric acid (mg/dl)	5.5 (2.3–9.4)
Fasting plasma glucose (mg/dl)	94 (62–120)
Fasting insulin (μ U/ml)	10.5 (0.4–55.5)
HOMA-IR	2.6 (0.1–12.5)
Treatment*	
PEG-IFN α -2b dose (μ g/kg)	1.4 (0.7–1.9)
Ribavirin dose (mg/kg)	11.0 (3.7–14.2)
Histological findings	
Stage of fibrosis (F1/F2/F3/ND)	53/32/20/18
Hepatocyte steatosis (<5% (Absent)/ \geq 5% (Present)/ND)	38/64/21
Amino acid substitutions in the HCV	
Core aa 70 (arginine/glutamine (histidine))	69/54
Core aa 91 (leucine/methionine)	71/52
ISDR of NS5A (wild-type/mutant-type/ND)	94/22/7

HOMA-IR, homeostasis model for assessment of insulin resistance; ND, not determined.

Data are number and percentages of patients, except those denoted by *, which represent the median (range) values.

based on histological assessment according to the scoring system of Desmet et al. [1994]. Hepatocyte steatosis was assessed as the percentage of hepatocytes containing fat droplet, and subjects were considered to have steatosis in the presence of fat droplets in \geq 5% of hepatocytes.

Diagnosis of Liver Cirrhosis, Insulin Resistance, and Diabetes Mellitus

Liver cirrhosis was diagnosed based on the presence of markedly irregular surface with nodular formation in the liver, evident on peritoneoscopy, histological assessment according to the scoring system of Desmet et al. [1994], or on computed tomography or ultrasonography. Ascites, edema, and esophageal varicosities, facilitated the diagnosis when present.

The diagnosis of type 2 diabetes was based on the revised criteria of the American Diabetes Association using a value of fasting plasma glucose of \geq 126 mg/dl on at least two occasions [American Diabetes Association, 2000]. IR was assessed by the Homeostasis Model for Assessment of Insulin Resistance (HOMA-IR) method

[Matthews et al., 1985], using the following equation: $\text{HOMA-IR} = \text{fasting plasma glucose (mg/dl)} \times \text{fasting insulin } (\mu\text{U/ml}) / 405$. HOMA-IR values of \geq 2.5, and \geq 3.5 were evaluated as IR, and severe IR, respectively.

Detection of Amino Acid Substitutions in Core Region and NS5A Region

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of genotype 1b was determined and then compared with the consensus sequence constructed on 50 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2209–2248 aa in the NS5A of genotype 1b (IFN-sensitivity determining region [ISDR]) reported by Enomoto et al. [1995, 1996] was also determined, and the numbers of aa substitutions in ISDR were defined as wild-type (\leq 1) or mutant-type (\geq 2).

In the present study, aa substitutions of the core region and NS5A-ISDR were analyzed by direct

sequencing [Enomoto et al., 1995, 1996; Akuta et al., 2005]. 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, Japan). 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°C for 15 min. The 35 cycles of amplification were set as follows: denaturation for 1 min at 94°C, annealing of primers for 2 min at 55°C, and extension for 3 min at 72°C with an additional 7 min for extension. Then 1 µl of the first PCR product was transferred to the second PCR reaction. 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, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

Statistical Analysis

Non-parametric tests were used to compare variables between groups, including the Mann-Whitney *U* test, chi-squared test, and Fisher's exact probability test. Univariate and multivariate logistic regression analyses were used to determine the independent predictive factors of IR. The odds ratios and 95% confidence intervals (95%CI) were also calculated. All *P*-values <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance (*P* <0.05) or marginal significance (*P* <0.10) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent factors. Potential predictive factors of IR included the following pretreatment variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), ALT, albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet, count, indocyanine green retention rate at 15 min (ICG R15), serum iron, serum ferritin, creatinine clearance, level of viremia, alfa-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol,

triglycerides, uric acid, fasting plasma glucose, fasting insulin, HOMA-IR values, stage of fibrosis, hepatocyte steatosis, PEG-IFN dose/body weight, RBV dose/body weight, and aa substitution in the core and ISDR of NS5A. Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL).

RESULTS

HOMA-IR Values of Patients Infected With HCV-1b Without Cirrhosis and Diabetes Mellitus

As a whole, 16.3% (20 of 123 patients), 32.5% (40 of 123), 23.6% (29 of 123), and 27.6% (34 of 123) indicated HOMA-IR values of ≤1.4, 1.5–2.4, 2.5–3.4, and ≥3.5, respectively (Fig. 1). Thus, patients with IR (HOMA-IR ≥2.5) were present in 51.2% (63 of 123), and exceeded 50%. Furthermore, patients with severe IR (HOMA-IR ≥3.5) were present in 27.6%. These results show that patients, infected with HCV-1b without cirrhosis and diabetes mellitus, might indicate IR frequently.

Factors Associated With Insulin Resistance in Univariate and Multivariate Analyses

The whole population sample of 123 patients were analyzed to determine factors that could be associated with IR. IR (HOMA-IR ≥2.5) was detected in 63 of 123 (51.2%) patients. Univariate analysis identified six parameters that tended to or significantly influenced IR. These included age (≥55 years, *P* = 0.072), body mass index (≥25 kg/m², *P* < 0.001), serum ferritin (≥200 µg/L, *P* = 0.071), family history of liver disease (Absent, *P* = 0.077), hepatocyte steatosis (Present (≥5%), *P* = 0.002), and aa substitutions of the core region (Gln70 (His70) and/or Met91, *P* = 0.092). Multivariate analysis identified two parameters that independently influenced IR, including body mass index (≥25 kg/m², *P* = 0.001) and hepatocyte steatosis (Present (≥5%), *P* = 0.028).

Severe IR (HOMA-IR ≥3.5) was detected in 34 of 123 (27.6%) patients. Univariate analysis identified five

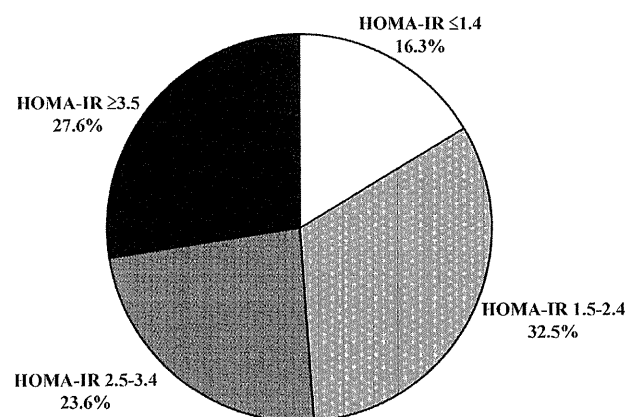


Fig. 1. HOMA-IR values of patients infected with HCV genotype 1b without cirrhosis and diabetes mellitus. As a whole, 16.3%, 32.5%, 23.6%, and 27.6% indicated HOMA-IR values of ≤1.4, 1.5–2.4, 2.5–3.4, and ≥3.5, respectively. These results show that patients, infected with HCV genotype 1b without cirrhosis and diabetes mellitus, might indicate IR frequently.

TABLE II. Factors Associated With Severe IR (HOMA-IR ≥ 3.5) in Patients Infected With HCV Genotype 1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Hepatocyte steatosis	1: Absent (<5%)	1	0.021
	2: Present ($\geq 5\%$)	4.170 (1.235–14.08)	
Substitution of aa 70 and 91	1: Arg70 and Leu91	1	0.021
	2: Gln70 (His70) and/or Met91	3.654 (1.215–10.99)	
Age (years)	1: <55	1	0.037
	2: ≥ 55	3.015 (1.071–8.488)	

parameters that tended to or significantly influenced severe IR. These included age (≥ 55 years, $P=0.015$), body mass index (≥ 25 kg/m², $P=0.025$), hepatocyte steatosis (Present ($\geq 5\%$), $P=0.003$), triglycerides (≥ 100 mg/dl, $P=0.060$), and aa substitutions of the core region (Gln70 (His70) and/or Met91, $P=0.020$). Multivariate analysis identified three parameters that independently influenced severe IR, including hepatocyte steatosis (Present ($\geq 5\%$), $P=0.021$), aa substitutions of the core region (Gln70 (His70) and/or Met91, $P=0.021$), and age (≥ 55 years, $P=0.037$) (Table II).

aa Substitutions of Core Region and HOMA-IR Values

The entire population sample was also analyzed to determine the relationship between aa substitutions of the core region and HOMA-IR values. HOMA-IR values of 81 patients with Gln70 (His70) and/or Met91 (median; 2.9) indicated the higher levels than those of 42 patients with Arg70 and Leu91 (median; 2.3), significantly ($P=0.022$) (Fig. 2).

Furthermore, the proportions of patients with Gln70 (His70) and/or Met91 among those with HOMA-IR values of ≤ 1.4 , 1.5–2.4, 2.5–3.4, 3.5–3.9, and ≥ 4.0 were 60.0% (12 of 20 patients), 57.5% (23 of 40), 62.1% (18 of 29), 83.3% (5 of 6), and 82.1% (23 of 28), respectively

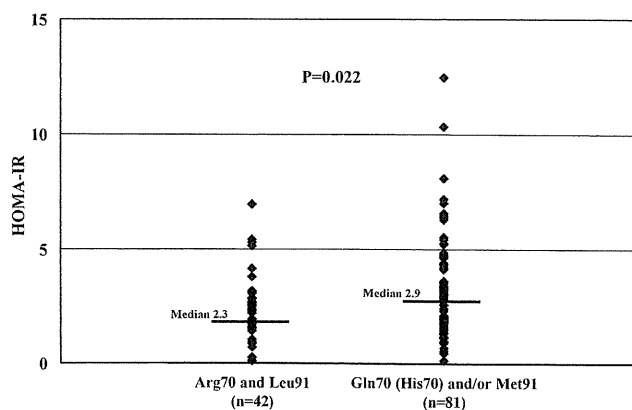


Fig. 2. aa substitutions of HCV core region and HOMA-IR values. HOMA-IR values of 81 patients with Gln70 (His70) and/or Met91 indicated the higher levels than those of 42 patients with Arg70 and Leu91, significantly ($P=0.022$).

(Fig. 3). Thus, the higher the proportion of patients with Gln70 (His70) and/or Met91, the higher HOMA-IR values, and significantly lower proportions of patients with Gln70 (His70) and/or Met91 were noted among those without severe IR (59.6% (53 of 89)) than those with severe IR (82.4% (28 of 34)) ($P=0.020$).

Treatment Efficacy of PEG-IFN Plus RBV Combination Therapy According to HOMA-IR Values

Of the 123 patients, 72 could be evaluated as 48-week regimen of PEG-IFN plus RBV combination therapy. Seventy-two patients received PEG-IFN α -2b combination therapy at a median dose of 1.5 μ g/kg (range, 0.8–1.8 μ g/kg) subcutaneously each week plus oral RBV at a median dose of 11.3 mg/kg (range, 9.7–14.2 mg/kg) daily for 48 weeks. 51.4% (37 of 72 patients) could achieve SVR, and 25.0% (18 of 72) had NVR.

In each groups with IR or without IR, SVR was achieved by 19 of 38 patients (50.0%) and 18 of 34 (52.9%), respectively. The proportions of SVR in group with IR was not significantly different from those in group without IR. Furthermore, in each groups with IR or without IR, NVR was identified in 11 of 38 patients (28.9%) and 7 of 34 (20.6%), respectively. The proportions of NVR in group with IR was not significantly different from those in group without IR.

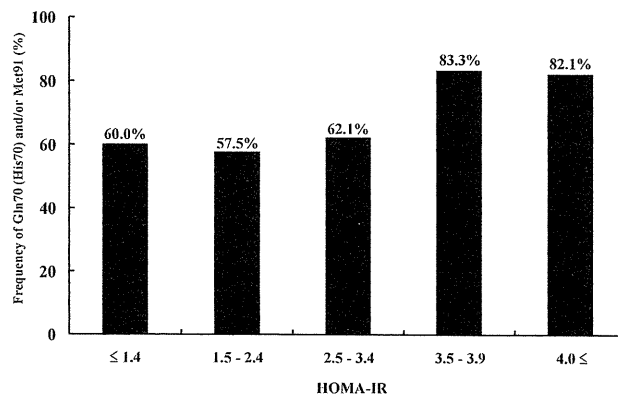


Fig. 3. The proportions of patients with Gln70 (His70) and/or Met91 and HOMA-IR values. Higher frequencies of Gln70 (His70) and/or Met91 correlated with higher HOMA-IR values. Significantly lower proportions of patients with Gln70 (His70) and/or Met91 were noted among those without severe IR (HOMA-IR < 3.5) (59.6%) than those with severe IR (HOMA-IR ≥ 3.5) (82.4%) ($P=0.020$).

In each groups with severe IR or without severe IR, SVR was achieved by 11 of 20 patients (55.0%) and 26 of 52 (50.0%), respectively. The proportions of SVR in group with severe IR was not significantly different from those in group without severe IR. Furthermore, in each groups with severe IR or without severe IR, NVR was identified in 6 of 20 patients (30.0%) and 12 of 52 (23.1%), respectively. The proportions of NVR in group with severe IR was not significantly different from those in group without severe IR.

In this study, HOMA-IR values were not useful as pretreatment predictors of 48-week PEG-IFN plus RBV combination therapy in HCV-1b patients without cirrhosis and diabetes mellitus.

DISCUSSION

Shintani et al. [2004] reported that HCV core protein induced IR in transgenic mice, and provided a direct experimental evidence for the contribution of HCV core protein in the development of IR in human HCV infection. The results of the present study showed that higher frequencies of Gln70 (His70) and/or Met91 in HCV-1b core region might correlated with higher HOMA-IR values. Thus, the present results supported the potential of core region in the development of IR, and clinically linked substitutions of aa 70 and/or 91 in HCV-1b core region to IR. Especially, these findings without diabetes mellitus and cirrhosis suggest that the real connection between IR and HCV-1b infection is initiated at early stages of liver disease. The limitations of the present study were that it could not investigate an improvement of IR in patients who developed the viral eradication after antiviral treatment [Kawaguchi et al., 2007; Arase et al., 2008], as a direct evidence for the contribution of aa substitutions in HCV-1b core region. Further studies that examine the structural and functional impact of aa substitutions should be conducted to confirm the above finding.

To our knowledge, the present study is first report to identify the factors associated with IR of patients without diabetes mellitus and cirrhosis infected with HCV-1b. Especially, multivariate analysis identified age (≥ 55 years), body mass index (≥ 25 kg/m²), hepatocyte steatosis (Present ($\geq 5\%$)), and aa substitutions of the core region (Gln70 (His70) and/or Met91) as significant determinants of IR (HOMA-IR ≥ 2.5) and/or severe IR (HOMA-IR ≥ 3.5). However, this study identified aa substitutions of the core region as significant determinants of severe IR, and did not identify as determinants of IR. The discrepant results may be due to one or more factors. The first reason for this is probably the small number of patients in the present study (e.g., possible type II error). Univariate analysis really identified aa substitutions of the core region that tended to influence IR. Furthermore, even if HOMA-IR values were also divided into two groups of ≥ 3.0 and ≤ 2.9 , multivariate analysis identified aa substitutions of the core region as significant determinants of ≥ 3.0 (data not

shown). Hence, further studies based on the large number of patients should be performed in the future. The second reason is probably the difference of objects, based on HCV-1b patients without diabetes mellitus and cirrhosis. Previous report indicated that HCV-related diabetes mellitus might occur in association with IR, hepatocyte steatosis, and high levels of both tumor-necrosis factor and CXCL10 [Antonelli et al., 2009], so patients with severe IR do not always have diabetes mellitus. However, IR develops type 2 diabetes mellitus as its major late feature, and is also associated with advanced fibrosis [Petrides et al., 1994; Petta et al., 2008]. Hence, the biological mechanisms underlying the association between HCV core region and IR are probably multifactorial, and the present study based on patients without diabetes mellitus and cirrhosis as confounding factors, that might affect IR, are very important for estimating the true relationship between HCV core region and IR. The present study is first report to identified aa substitutions of the core region (Gln70 (His70) and/or Met91) as significant determinants of severe IR, in HCV-1b patients without cirrhosis and diabetes mellitus.

Moriya et al. [1998] reported that HCV core protein induced HCC in transgenic mice, and provided a direct experimental evidence for the contribution of HCV core protein in the development of HCC in human HCV infection. Previous reports supported the oncogenic potential of the HCV core region and clinically linked substitutions of aa 70 and/or 91 in HCV-1b core region to HCC [Akuta et al., 2007d, 2008]. IR and glucose metabolism impairment are associated with HCC [El-Serag et al., 2001; Lai et al., 2006; Veldt et al., 2008]. The present study suggested the presence of IR-dependent pathway as a mechanism of HCV-1b core region-associated hepatocarcinogenesis, and the importance of eradication of the virus with Gln70 (His70) and/or Met91 in reducing the development of HCC through this pathway.

Treatment efficacy of 48-week PEG-IFN plus RBV combination therapy according to HOMA-IR values is controversial. Chu et al. [2008] reported that IR was a major determinant of SVR in HCV-1 patients receiving PEG-IFN plus RBV, but treatment duration was 24 weeks. Georgescu et al. [2008] reported that high HOMA-IR values could not affect treatment efficacy of 48-week PEG-IFN plus RBV therapy in HCV-1 patients, after excluding the patients of metabolic syndrome criteria. The present study based on HCV-1b patients without cirrhosis and diabetes mellitus also showed that HOMA-IR values might be not useful as predictors of 48-week PEG-IFN plus RBV therapy. This reason is probably related to exclude patients of diabetes mellitus as one of metabolic syndrome criteria, and the results might support the previous report of Georgescu et al. [2008]. To our knowledge, the present study is first report to investigate the relation between HOMA-IR values and treatment efficacy of HCV-1b patients, especially without cirrhosis and diabetes mellitus, receiving 48-week PEG-IFN plus RBV combination

therapy. Further studies based on the large number of patients should be performed in the future.

In conclusion, the results of the present study indicated that substitutions of HCV-1b core region were the important predictor of severe IR in patients without cirrhosis and diabetes mellitus. This finding highlights the importance of eradication of the virus with Gln70 (His70) and/or Met91 in reducing the development of severe IR. The limitations of the present study were that it did not investigate other genotypes apart from HCV-1b, the geographic diversities of HCV-1b core region (distribution of Arg70 or Gln70 (His70), and Leu91 or Met91), 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 meaning of higher HOMA-IR values in HCV infection.

REFERENCES

- Akuta N, Chayama K, Suzuki F, Someya T, Kobayashi M, Tsubota A, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kumada H. 2001. Risk factors of hepatitis C virus-related liver cirrhosis in young adults: Positive family history of liver disease and transporter associated with antigen processing 2 (TAP2) *0201 allele. *J Med Virol* 64:109–116.
- Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 48:372–380.
- Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2006. Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* 78:83–90.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007a. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: Amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 46:403–410.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007b. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol* 79:1686–1695.
- 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. 2007c. 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* 50:361–368.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007d. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 46:1357–1364.
- Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2008. Substitution of amino acid 70 in the hepatitis C virus core region of genotype 1b is an important predictor of elevated alpha-fetoprotein in patients without hepatocellular carcinoma. *J Med Virol* 80:1354–1362.
- Allison ME, Wreghitt T, Palmer CR, Alexander GJ. 1994. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 21:1135–1139.
- American Diabetes Association. 2000. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. American Diabetes Association: Clinical Practice Recommendations 2000 Committee Report. *Diabetes Care* 23:S4–S19.
- Antonelli A, Ferri C, Fallahi P, Pampana A, Ferrari SM, Goglia F, Ferrannini E. 2005. Hepatitis C virus infection: Evidence for an association with type 2 diabetes. *Diabetes Care* 28:2548–2550.
- Antonelli A, Ferri C, Ferrari SM, Colaci M, Sansonno D, Fallahi P. 2009. Endocrine manifestations of hepatitis C virus infection. *Nat Clin Pract Endocrinol Metab* 5:26–34.
- Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Hirakawa M, Ikeda K, Kumada H. 2008. Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. *Hepatology* (in press).
- Cammà C, Bruno S, Di Marco V, Di Bona D, Rumi M, Vinci M, Rebusci C, Cividini A, Pizzolanti G, Minola E, Mondelli MU, Colombo M, Pinzello G, Craxi A. 2006. Insulin resistance is associated with steatosis in nondiabetic patients with genotype 1 chronic hepatitis C. *Hepatology* 43:64–71.
- Caronia S, Taylor K, Pagliaro L, Carr C, Palazzo U, Petrik J, O'Rahilly S, Shore S, Tom BD, Alexander GJ. 1999. Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 30:1059–1063.
- Chayama K, Tsubota A, Arase Y, Saitoh S, Koida I, Ikeda K, Matsumoto T, Kobayashi M, Iwasaki S, Koyama S, Morinaga T, Kumada H. 1993. Genotypic subtyping of hepatitis C virus. *J Gastroenterol Hepatol* 8:150–156.
- Chu CJ, Lee SD, Hung TH, Lin HC, Hwang SJ, Lee FY, Lu RH, Yu MI, Chang CY, Yang PL, Lee CY, Chang FY. 2008. Insulin resistance is a major determinant of sustained virological response in genotype 1 chronic hepatitis C patients receiving peginterferon alpha-2b plus ribavirin. *Aliment Pharmacol Ther* 29:46–54.
- Conjeevaram HS, Kleiner DE, Everhart JE, Hoofnagle JH, Zacks S, Afzal NH, Wahed AS. Virahep-C Study Group. 2007. Race, insulin resistance and hepatic steatosis in chronic hepatitis C. *Hepatology* 45:80–87.
- Desmet VJ, Gerber M, Hoofnagle JH, Manna M, Scheuer PJ. 1994. Classification of chronic hepatitis: Diagnosis, grading and staging. *Hepatology* 19:1513–1520.
- Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE. 2007. Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. *J Virol* 81:8211–8224.
- D'Souza R, Sabin CA, Foster GR. 2005. Insulin resistance plays a significant role in liver fibrosis in chronic hepatitis C and in the response to antiviral therapy. *Am J Gastroenterol* 100:1509–1515.
- Dusheiko GM. 1998. The natural course of chronic hepatitis C: Implications for clinical practice. *J Viral Hepatol Suppl* 1:9–12.
- El-Serag HB, Richardson PA, Everhart JE. 2001. The role of diabetes in hepatocellular carcinoma: A case-control study among United States Veterans. *Am J Gastroenterol* 96:2462–2467.
- Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C. 1995. Comparison of full-length sequences of interferon sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. *J Clin Invest* 96:224–230.
- Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 334:77–81.
- Fartoux L, Poujol-Robert A, Guéhot J, Wendum D, Poupon R, Serfaty L. 2005. Insulin resistance is a cause of steatosis and fibrosis progression in chronic hepatitis C. *Gut* 54:1003–1008.
- Georgescu EF, Lonescu R, Florescu G, Mateescu G, Vancica L. 2008. Steatosis, insulin resistance, iron overload, fibrosis and viral load as negative factors affecting early (EVR) and sustained (SVR) virological response in patients with chronic hepatitis C treated with peginterferon and ribavirin. *Hepatology* 48:A869.
- Hickman LJ, Powell EE, Prins JB, Clouston AD, Ash S, Purdie DM, Jonsson JR. 2003. In overweight patients with chronic hepatitis C, circulating insulin is associated with hepatic fibrosis: Implications for therapy. *J Hepatol* 39:1042–1048.
- Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. 2003. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. *Gastroenterology* 125:1695–1704.

- Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Koida I, Arase Y, Fukuda M, Chayama K, Murashima N, Kumada H. 1998. Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: A prospective observation of 2215 patients. *J Hepatol* 28:930–938.
- Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimoto K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci USA* 87:9524–9528.
- Kawaguchi T, Ide T, Taniguchi E, Hirano E, Itou M, Sumie S, Nagao Y, Yanagimoto C, Hanada S, Koga H, Sata M. 2007. Clearance of HCV improves insulin resistance, beta-cell function, and hepatic expression of insulin receptor substrate 1 and 2. *Am J Gastroenterol* 102:570–576.
- Kenny-Walsh E. 1999. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med* 340:1228–1233.
- Lai MS, Hsieh MS, Chiu YH, Chen TH. 2006. Type 2 diabetes and hepatocellular carcinoma: A cohort study in high prevalence area of hepatitis virus infection. *Hepatology* 43:1295–1302.
- Lecube A, Hernández C, Genescà J, Esteban JI, Jardí R, Simó R. 2004. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: A multivariate analysis considering the liver injury. *Diabetes Care* 27:1171–1175.
- Lecube A, Hernández C, Genescà J, Simó R. 2006. Proinflammatory cytokines, insulin resistance, and insulin secretion in chronic hepatitis C patients: A case-control study. *Diabetes Care* 29:1096–1101.
- Mason AL, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP. 1999. Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 29:328–333.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419.
- Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. 2000. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 133:592–599.
- Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, Szklo M, Thomas DL. 2003. Hepatitis C virus infection and incident type 2 diabetes. *Hepatology* 38:50–56.
- Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. 1998. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med* 4:1065–1067.
- Moucarri R, Asselah T, Cazals-Hatem D, Voitot H, Boyer N, Ripault MP, Sobesky R, Martinot-Peignoux M, Maylin S, Nicolas-Chanoine MH, Paradis V, Vidaud M, Valla D, Bedossa P, Marcellin P. 2008. Insulin resistance in chronic hepatitis C: Association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. *Gastroenterology* 134:416–423.
- Niederer C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D. 1998. Progress of chronic hepatitis C: Results of a large, prospective cohort study. *Hepatology* 28:1687–1695.
- Okanoue T, Itoh Y, Yotsuyanagi H, Tanaka E, Yoshioka K, Izumi N, Kumada H. 2008. Substitution of core amino acid 91 lowers rapid virological response and substitution of core amino 70 lowers sustained virological response to peginterferon alfa-2b plus ribavirin in chronic hepatitis C patients with genotype 1b-nationwide study. *Hepatology* 48:868A.
- Petrides AS, Vogt C, Schulze-Berge D, Matthews D, Strohmeier G. 1994. Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. *Hepatology* 19:616–627.
- Petta S, Cammà C, Marco VD, Alessi N, Cabibi D, Caldarella R, Licata A, Massenti F, Tarantino G, Marchesini G, Craxi A. 2008. Insulin resistance and diabetes increase fibrosis in the liver of patients with genotype 1 HCV infection. *Am J Gastroenterol* 103:1136–1144.
- Romero-Gómez M, Del Mar Viloria M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, Corpas R, Cruz M, Grande L, Vázquez L, Muñoz-De-Rueda P, López-Serrano P, Gila A, Gutiérrez ML, Pérez C, Ruiz-Extremera A, Suárez E, Castillo J. 2005. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 128:636–641.
- Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. 2004. Hepatitis C virus infection and diabetes: Direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 126:840–848.
- Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, de Knegt RJ, Zeuzem S, Manns MP, Hansen BE, Schalm SW, Janssen HL. 2008. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 47:1856–1862.
- Zein NN, Abdulkarim AS, Wiesner RH, Egan KS, Persing DH. 2000. Prevalence of diabetes mellitus in patients with end-stage liver cirrhosis due to hepatitis C, alcohol, or cholestatic disease. *J Hepatol* 32:209–217.
- Zein CO, Levy C, Basu A, Zein NN. 2005. Chronic hepatitis C and type II diabetes mellitus: A prospective cross-sectional study. *Am J Gastroenterol* 100:48–55.

Sustained Virological Response Reduces Incidence of Onset of Type 2 Diabetes in Chronic Hepatitis C

Yasuji Arase, Fumitaka Suzuki, Yoshiyuki Suzuki, Norio Akuta, Masahiro Kobayashi, Yusuke Kawamura, Hiromi Yatsuji, Hitomi Sezaki, Tetsuya Hosaka, Miharuru Hirakawa, Kenji Ikeda, and Hiromitsu Kumada

Diabetes is present in patients with chronic hepatitis C virus infection. The aim of this retrospective cohort study was to assess the cumulative development incidence and predictive factors for type 2 diabetes after the termination of interferon therapy in Japanese patients positive for hepatitis C virus (HCV). A total of 2,842 HCV-positive patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin were enrolled. The mean observation period was 6.4 years. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses during follow-up. The primary goal was the onset of type 2 diabetes. Evaluation was performed by using the Kaplan-Meier method and Cox proportional hazard analysis. Of 2,842 HCV patients, 143 patients developed type 2 diabetes. The cumulative development rate of type 2 diabetes was 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years. Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), the patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was ≥ 50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). **Conclusion:** Our results indicate sustained virological response causes a two-thirds reduction in the risk of type 2 diabetes development in HCV-positive patients treated with IFN. (HEPATOLOGY 2009;49:739-744.)

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease in world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20% to 50% of cases over a period of 10 to 30 years.¹⁻³ In addition, HCV is a major risk for hepatocellular carcinoma (HCC).⁴⁻⁸ Moreover, chronic HCV infection has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, porphyria cutanea tarda, membranoproliferative glomerulonephritis, autoimmune thyroid-

itis, sialadenitis, and cardiomyopathy.⁹⁻¹³ Lately, data supporting a link between type 2 diabetes mellitus (T2DM) and chronic hepatitis C infection have been reported.^{14,15}

Although there is growing evidence to support the concept that HCV infection is a risk factor for developing T2DM, there have been a few interventional studies confirming this issue. This issue needs to be confirmed with a long-term follow-up of patients with high risk of developing diabetes. Thus, prospective studies including metabolic evaluations are clearly needed to clarify these issues.

With this background in mind, the cohort study was initiated to investigate the cumulative incidence and risk factors of T2DM after prolonged follow-up in HCV-infected patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Patients and Methods

Patients. There were 5,890 patients diagnosed with chronic HCV infection and treated with IFN mono-

Abbreviations: CI, confidence interval; FPG, fasting plasma glucose; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response; T2DM, type 2 diabetes mellitus.

From the Department of Hepatology, Toranomon Hospital, Tokyo, Japan.

Received September 25, 2008; accepted October 13, 2008.

Supported in part by grants-in-aid from Okinaka Memorial Institute for Medical Research and the Japanese Ministry of Health, Labor, and Welfare.

Address reprint requests to: Yasuji Arase, M.D., Department of Hepatology, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo 105-8470, Japan. E-mail: es9y-ars@asahi-net.or.jp; fax: (81)-3-3582-7068.

Copyright © 2008 by the American Association for the Study of Liver Diseases.

Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.22703

Potential conflict of interest: Nothing to report.

therapy or combination IFN + ribavirin therapy between September 1990 and March 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these, 2,842 patients satisfied the following criteria: (1) no evidence of diabetes mellitus for 3 months after the termination of IFN (plasma glucose concentration <126 mg/dL [6.9 mmol/L] in the fasting state, <200 mg/dL [11.0 mmol/L] in casual state and/or 2 hours after a 75-g oral glucose load); (2) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy before the initiation of IFN therapy; (3) positivity for serum HCV RNA before the initiation of IFN therapy; (4) period of ≤ 1 year of IFN therapy; (5) negativity for hepatitis B surface antigen (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined via radioimmunoassay or spot hybridization; (6) no evidence of HCC nodules as shown on ultrasonography and/or computed tomography; and (7) no underlying systemic disease, such as systemic lupus erythematosus or rheumatic arthritis.

Patients who were taking medications known to alter glucose tolerance or had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial were excluded from the study. Patients were classified as having normal glucose or pre-diabetes based on fasting plasma glucose (FPG), casual plasma glucose, or 2-hour plasma glucose. The normal glucose group was regarded as having an FPG of <100 mg/dL, casual plasma glucose of <140 mg/dL, and/or 2-hour plasma glucose of <140 mg/dL. The pre-diabetes group was regarded as having an FPG of 100-125 mg/dL, casual plasma glucose of 140-200 mg/dL, and/or 2-hour plasma glucose of 140-200 mg/dL.¹⁶

Next, we assessed predictive factors for T2DM in chronic hepatitis C patients treated with IFN. The physicians in charge explained the purpose and method of this clinical trial to each patient and/or the patient's family. Informed consent was obtained from all living patients included in the present cohort study. The study was approved by the Institutional Review Board of our hospital.

Outcome Measures. The primary outcome was T2DM, diagnosed by the use of the 2003 criteria of the American Diabetes Association.¹⁶ These criteria include (1) casual plasma glucose ≥ 200 mg/dL; (2) FPG ≥ 126 mg/dL; (3) 2-hour post-glucose (oral glucose tolerance test) ≥ 200 mg/dL.

Laboratory Investigation. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan). Hepatitis B surface antigen was tested via radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored at

-80°C at the first consultation. Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Height and weight were recorded at baseline, and the body mass index was calculated as weight (in kg)/height (in m^2).

Evaluation of Liver Cirrhosis. Liver status of the 2,842 patients was mainly determined via peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.¹⁷

Follow-up. The starting time of follow-up was 3 months after the termination of IFN therapy. After that, patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check-up. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses. These included aminotransferase activities, total cholesterol, platelet counts, and serum HCV RNA level. Three hundred twenty-four patients were lost to follow-up; because the appearance of T2DM and death was not identified in these patients, they were considered as censored data in the statistical analysis.¹⁸ Moreover, patients retreated with antiviral agents were regarded as withdrawals at the time of starting the retreatment of antiviral agents.

Statistical Analysis. The cumulative appearance rate of T2DM was calculated from 3 months after the termination of IFN treatment to the appearance of T2DM using the Kaplan-Meier method. Differences in the development of T2DM were tested using the log rank test. Independent factors associated with the incidence rate of T2DM were analyzed by the Cox proportional hazard model. The following 11 variables were analyzed for potential covariates for incidence of T2DM at the time of termination of IFN therapy at our hospital: age, sex, state of liver disease (chronic hepatitis or liver cirrhosis), body mass index, glucose level, aspartate aminotransferase level, alanine aminotransferase level, type of IFN, total dose of IFN, efficacy of IFN therapy, hypertension, triglyceride level, and total cholesterol level. A *P* value of less than 0.05 was considered significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

Results

Patient Characteristics. Table 1 shows the characteristics of the 2,842 HCV-positive patients treated with

Table 1. Patient Characteristics

N	2,842
Sex (male/female)	1,778/1,064
Age (years)	51.8 ± 9.0
Height (cm)	163.8 ± 9.1
Body weight (kg)	62.7 ± 11.7
Body mass index	23.3 ± 3.2
Blood pressure (systolic/diastolic, mm Hg)	128 ± 18/77 ± 12
HCV genotype (1b/2a/2b/other)	744/752/290/56
HCV RNA level (KIU/mL)	593 ± 540
Staging (non-LC/LC)	2,649/193
Blood glucose level (normal/prediabetes)	2,601/241
Fasting plasma glucose (mg/dL)	87 ± 24
Triglyceride (mg/dL)	166 ± 31
Total bilirubin (g/dL)	102 ± 56
AST (IU/L)	74 ± 63
ALT (IU/L)	116 ± 102
IFN monotherapy*/combination therapy†	2,417/425
Efficacy of treatment (SVR/non-SVR)	1,175/1,667
Follow-up period (years)	6.4 ± 5.0

Data are expressed as the number of patients or mean ± standard deviation.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LC, liver cirrhosis; SVR, sustained virological response.

*Outbreak of IFN monotherapy: recombinant IFN- α 2a, 304 cases; recombinant IFN- α 2b, 235 cases; natural IFN- β , 1,355 cases; natural IFN- β , 522 cases; total dose of IFN = 598 ± 170 MU.

†Outbreak of combination therapy: recombinant IFN- α 2b + ribavirin, 175 cases; total dose of IFN = 537 ± 196 MU; total dose of ribavirin = 182 ± 69 g; pegylated IFN- α 2b + ribavirin, 250 cases; total dose of pegylated IFN = 4.28 ± 1.17 mg; total dose of ribavirin = 232 ± 60 g.

IFN monotherapy or combination therapy with IFN and ribavirin. The sustained virological response (SVR) rate was 36.7% (886/2417) in IFN monotherapy and 68% (289/425) in IFN + ribavirin therapy. Thus, the number of patients with SVR was 1,175. The mean period after the termination of antiviral drugs was 6.4 years.

Incidence of T2DM in Patients with HCV. A total of 143 patients (102 men and 41 women) developed T2DM during a mean observation period of 6.4 years. Of these, 26 were SVR and 117 were non-SVR. The cumulative development rate of T2DM was determined to be 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years using the Kaplan-Meier method (Fig. 1). The factors associated with the incidence of T2DM in all 2,842 patients treated with IFN therapy are shown in Table 2.

Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was >50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). SVR causes a two-thirds reduction of development of T2DM in patients treated with IFN. In addition to SVR, age ≥ 50

years, liver cirrhosis, and pre-diabetes contribute to a high risk of developing diabetes. The cumulative development rates of T2DM based on difference of age, efficacy of the IFN therapy, histological diagnosis, and glucose level at the starting time of follow-up are shown in Fig. 2.

Fig. 3 shows the impact of reduction due to SVR on the incidence of T2DM in patients with ≥ 50 years, liver cirrhosis, or pre-diabetes. When patients with age ≥ 50 years, liver cirrhosis, and pre-diabetes have SVR after IFN therapy, SVR could statistically reduce the onset of T2DM compared with those without SVR.

Discussion

We have described the development incidence of diabetes after the termination of antiviral therapy in HCV-positive patients treated with IFN therapy in the present study. Diabetes has been reported in less than 0.08% of patients treated with IFN^{19,20}; thus, to exclude diabetes originating from IFN-related side effects, patients without diabetes for 3 months after the termination of IFN were enrolled in the present study. The present study indicates that the annual incidence of T2DM for a prolonged follow-up after the termination of IFN therapy among HCV patients is 0.8% to 1.0%. The present study was limited by a retrospective cohort trial. We started the present study in 1991 based on the diabetes mellitus criteria published by Fajans.²¹ However, after that, diabetes mellitus criteria were revised. We thus rechecked the diagnosis of T2DM based on the diabetes mellitus criteria of 2003 in patients seen prior to 2003.¹⁶ Because of rechecking the diagnosis of T2DM on the basis of diabetes mellitus criteria in 2003, the present study was regarded as a retrospective cohort study. However, the patients were

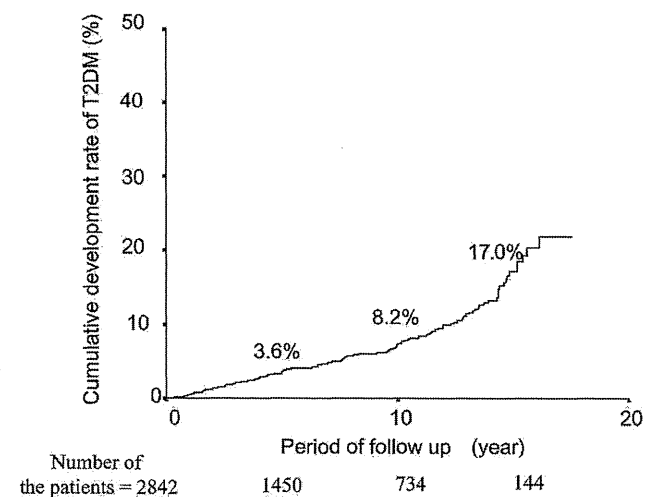


Fig. 1. Cumulative development rate of T2DM in patients treated with IFN.

Table 2. Predictive Factors for T2DM Development

Variables	Univariate Analysis		Cox Regression	
	HR (95% CI)	P Value	HR (95% CI)	P Value
Age, years (≥ 50 / <50)	2.55 (1.74-3.73)	<0.001	2.10 (1.38-3.18)	<0.001
Sex (female/male)	0.84 (0.59-1.19)	0.318		
Body mass index (≥ 25 / <25)	1.44 (0.98-2.08)	0.057		
HCV load (KIU/mL, $\geq 1,000$ / $<1,000$)	0.67 (0.43-1.03)	0.069		
Genotype (1/2)	0.73 (0.50-1.06)	0.098		
ALT (IU/L, ≥ 50 / <50)	1.83 (1.14-2.94)	0.012		
Glucose level (prediabetes/normal)	2.25 (1.53-3.33)	<0.0001	2.19 (1.43-3.37)	<0.001
Triglyceride (mg/dL, ≥ 150 / <150)	1.66 (0.93-2.98)	0.088		
Cholesterol (mg/dL, ≥ 220 / <220)	1.56 (0.62-3.95)	0.346		
Histological diagnosis (LC/non-LC)	4.03 (2.55-6.36)	<0.0001	3.30 (2.06-5.28)	<0.001
Combination of ribavirin (-/+)	1.53 (0.99-2.38)	0.058		
Type of IFN (α / β)	0.88 (0.57-1.35)	0.882		
Total dose of IFN (MU, ≥ 500 / <500)	0.91 (0.59-1.40)	0.672		
Efficacy (non-SVR/SVR)	2.73 (1.77-4.20)	<0.0001	2.78 (1.75-4.41)	<0.001

Data are expressed as the median (range).

Abbreviations: ALT, alanine aminotransferase; HR, hazard ratio; LC, liver cirrhosis.

prospectively followed. Another limitation of the study was that patients were treated with different types of antiviral therapy (IFN monotherapy or combination IFN + ribavirin therapy) for different duration (4 to 52 weeks).

This heterogeneity makes it difficult to interpret the results of the study. On the other hand, the strength of the present study is the long-term follow-up in the large numbers of patients included.

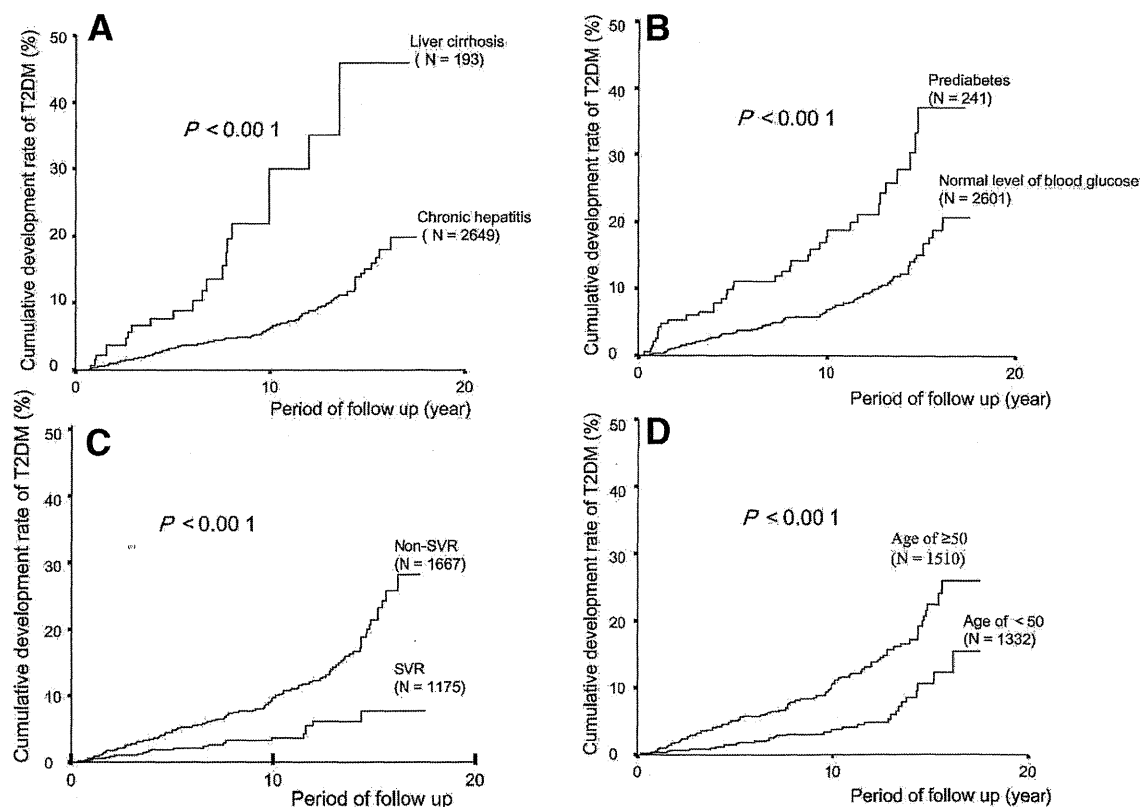


Fig. 2. Cumulative development rate of T2DM in patients treated with IFN. (A) Cumulative development rate of T2DM based on difference of hepatic fibrosis. (B) Cumulative development rate of T2DM based on the difference of glucose level. (C) Cumulative development rate of T2DM based on the difference of efficacy. (D) Cumulative development rate of T2DM based on the difference of age.

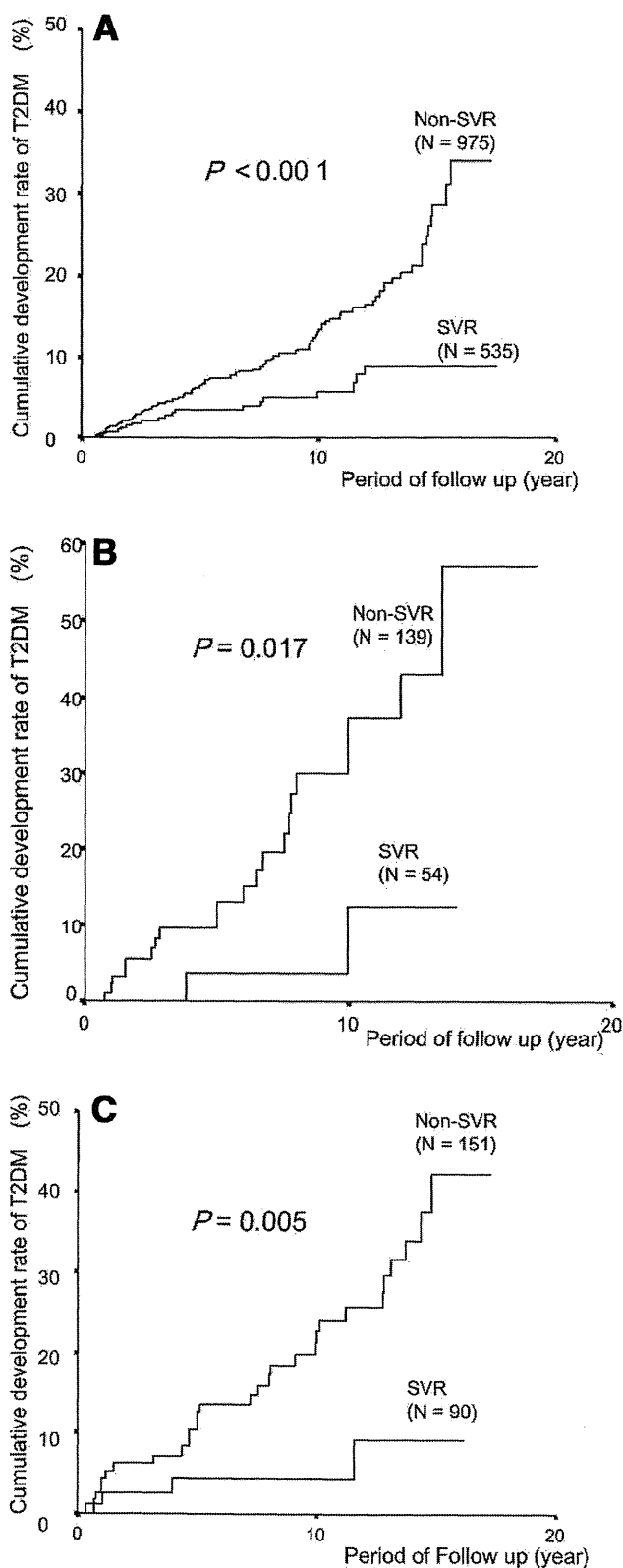


Fig. 3. Cumulative development rate of T2DM in patients with SVR or without SVR after IFN therapy. (A) Cumulative development rate of T2DM based on SVR or non-SVR in patients with age ≥ 50 years. (B) Cumulative development rate of T2DM based on SVR or non-SVR in patients with liver cirrhosis. (C) Cumulative development rate of T2DM based on the difference of SVR or non-SVR in patients with pre-diabetes.

The present study shows several findings with regard to development of T2DM after the termination of antiviral agents for HCV positive patients. First, the T2DM development rate in the non-SVR group was higher than that in the SVR group. The SVR caused a two-thirds reduction in the onset of T2DM in the course of posttreatment follow-up. That SVR reduced the onset of diabetes mellitus in HCV patients is in accordance with the data reported by Simó et al.²² and Romero-Gómez et al.²³ Though the role of HCV in the pathogenesis of diabetes mellitus remains speculative, the following possible mechanisms have been reported: (1) patients with HCV have a tendency to attain insulin resistance²⁴; (2) in transgenic mice, the expression of HCV core protein is associated with insulin resistance and T2DM development²⁵; and (3) SVR in HCV patients reduces insulin resistance and onset of the incidence of abnormal glucose value.²⁶ Thus, it is accepted that clearance of HCV reduces the onset of T2DM.

Second, in addition to persistence of HCV, the present study suggests that aging, histological progression, and pre-diabetes enhanced the onset of T2DM in patients with HCV infection. However, when HCV was eradicated even in patients with age ≥ 50 years, pre-diabetes, or liver cirrhosis, the cumulative development rate of T2DM decreased.

T2DM is increasing dramatically in many Asian nations, including Japan, over the past decades.²⁷ It is widely accepted that 7 to 8 million people are affected by diabetes mellitus in Japan. Approximately 8% to 10% of adults in Japan have T2DM. In general, T2DM is associated with a genetic predisposition, but it is also strongly influenced by lifestyle-related factors, such as eating habits and/or physical activity.²⁸⁻³³ The risk factors associated with T2DM include family history, age, sex, obesity, smoking, and physical activity. T2DM occurred in elderly patients compared to young patients. Life expectancies are long in Japan; thus, in the near future, a large number of patients with HCV will be >60 years of age. Therefore, it is apparent that the incidence of T2DM will increase in HCV-positive patients.

T2DM is a serious, costly disease. Treatment for T2DM may prevent some of its devastating complications, but does not usually restore normoglycemia or eliminate all the adverse consequences.^{28,29} Moreover, HCV patients with T2DM are at major risk for HCC.³⁴ On the efficacy of IFN therapy, it has been reported that T2DM reduces HCV eradication via combination IFN + ribavirin therapy.²⁶ Thus, it should be considered whether HCV-positive patients should be treated with antiviral drugs in the histological nonprogression stage and at a non-elderly age for prevention of T2DM onset. If

SVR obtained via antiviral therapy for HCV cannot only prevent progression to liver cirrhosis or HCC but also prevent the development of diabetes, the potential impact of IFN therapy is quite significant.

In conclusion, this retrospective study suggests that the annual incidence of T2DM among patients with HCV is 0.8% to 1.0%. Our results indicate that SVR causes a two-thirds reduction of T2DM development in HCV-positive patients treated with antiviral drugs.

Acknowledgment: The authors acknowledge the editorial assistance of Thomas Hughes.

References

- Kiyosawa K, Furuta S. Review of hepatitis C in Japan. *J Gastroenterol Hepatol* 1991;6:383-391.
- Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A, Alexander WJ, et al. The natural history of community acquired hepatitis C in the United States. *N Engl J Med* 1992;327:1899-1905.
- van Rossum TG, Vulto AG, de Man RA, Brouwer JT, Schalm SW. Review article: glycyrrhizin as a potential treatment for chronic hepatitis C. *Aliment Pharmacol Ther* 1998;12:199-205.
- Colombo M, Kuo G, Choo QL, Donato MF, Del Ninno E, Tommasini MA, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989;2:1006-1008.
- Hasan F, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, et al. Hepatitis C-associated hepatocellular carcinoma. *HEPATOLOGY* 1990;12:589-591.
- Kew MC, Houghton M, Choo QL, Kuo G. Hepatitis C virus antibodies in southern African blacks with hepatocellular carcinoma. *Lancet* 1990;335:873-874.
- Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993;328:1797-1801.
- Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, 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.
- Gumber SC, Chopra S. Hepatitis C: a multifaceted disease- review of extrahepatic manifestations. *Ann Intern Med* 1995;123:615-620.
- Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE, Hartwell P, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. *N Engl J Med* 1993;328:465-470.
- Pawlotsky JM, Roudot-Thoraval F, Simmonds P, Mellor J, Ben Yahia MB, André C, et al. Extrahepatic immunologic manifestations in chronic hepatitis C and hepatitis C virus serotypes. *Ann Intern Med* 1995;122:169-173.
- Boadas J, Rodriguez-Espinosa J, Enriquez J, Miralles F, Martínez-Cerezo FJ, González P, et al. Prevalence of thyroid autoantibodies is not increased in blood donors with hepatitis C virus infection. *J Hepatol* 1995;22:611-615.
- Agnello V, Chung RT, Kaplan LM. A role for hepatitis C virus infection in type II cryoglobulinemia. *N Engl J Med* 1992;327:1490-1495.
- Imazeki F, Yokosuka O, Fukai K, Kanda T, Kojima H, Saisho H. Prevalence of diabetes mellitus and insulin resistance in patients with chronic hepatitis C: comparison with hepatitis B virus-infected and hepatitis C virus-cleared patients. *Liver Int* 2008;28:355-362.
- Arao M, Murase K, Kusakabe A, Yoshioka K, Fukuzawa Y, Ishikawa T, et al. Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. *J Gastroenterol* 2003;38:355-360.
- Genuth S, Alberti KG, Bennett P, Buse J, Defronzo R, Kahn R, et al. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003;26:3160-3167.
- Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *HEPATOLOGY* 1994;19:1513-1520.
- Fleming TR, Harrington DP, O'Brien PC. Designs for group sequential tests. *Control Clin Trials* 1984;5:348-361.
- Fabris P, Betterle C, Greggio NA, Zanchetta R, Bosi E, Biasin MR, et al. Insulin-dependent diabetes mellitus during alpha-interferon therapy for chronic viral hepatitis. *J Hepatol* 1998;28:514-517.
- di Cesare E, Previti M, Russo F, Brancatelli S, Ingemi MC, Scoglio R, et al. Interferon-alpha therapy may induce insulin autoantibody development in patients with chronic viral hepatitis. *Dig Dis Sci* 1996;41:1672-1677.
- Fajans S. Classification and diagnosis of diabetes. In: Rifkin H, Porte D, eds. *Diabetes Mellitus: Theory and Practice*. 4th ed. New York: Elsevier, 1990:346-356.
- Simó R, Lecube A, Genesà J, Esteban JI, Hernández C. Sustained virological response correlates with reduction in the incidence of glucose abnormalities in patients with chronic hepatitis C virus infection. *Diabetes Care* 2006;29:2462-2466.
- Romero-Gómez M, Fernández-Rodríguez CM, Andrade RJ, Diago M, Alonso S, Planas R, et al. Effect of sustained virological response to treatment on the incidence of abnormal glucose values in chronic hepatitis C. *J Hepatol* 2008;48:721-727.
- Romero-Gómez M. Insulin resistance and hepatitis C. *World J Gastroenterol* 2006;12:7075-7080.
- Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004;126:840-848.
- Romero-Gómez M, Del Mar Vilorio M, Andrade RJ, Salmerón J, Diago M, Fernández-Rodríguez CM, et al. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005;128:636-641.
- Waki K, Noda M, Sasaki S, Matsumura Y, Takahashi Y, Isogawa A, et al. Alcohol consumption and other risk factors for self-reported diabetes among middle-aged Japanese: a population-based prospective study in the JPHC study cohort I. *Diabet Med* 2005;22:323-331.
- Thuluvath PJ, John PR. Association between hepatitis C, diabetes mellitus, and race. a case-control study. *Am J Gastroenterol* 2003;98:438-441.
- Mehta SH, Brancati FL, Strathdee SA, Pankow JS, Netski D, Coresh J, et al. Hepatitis C virus infection and incident type 2 diabetes. *HEPATOLOGY* 2003;38:50-56.
- Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004;27:1047-1053.
- UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; 352:837-853.
- UK Prospective Diabetes Study (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet* 1998;352:854-865.
- Elbein SC. The search for genes for type 2 diabetes in the post-genome era. *Endocrinology* 2002;143:2012-2018.
- Veldt BJ, Chen W, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, et al. Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *HEPATOLOGY* 2008;47:1856-1862.

Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C Patients with Genotype 1b and Low-virus Load

Yasuji Arase¹, Fumitaka Suzuki¹, Norio Akuta¹, Hitomi Sezaki¹, Yoshiyuki Suzuki¹, Yusuke Kawamura¹, Masahiro Kobayashi¹, Tetsuya Hosaka¹, Hiromi Yatsuji¹, Miharuru Hirakawa¹, Satoshi Saito¹, Kenji Ikeda¹, Mariko Kobayashi² and Hiromitsu Kumada¹

Abstract

Objective The aim of this study was to evaluate the efficacy of combination therapy of peginterferon and ribavirin in patients infected with hepatitis C virus (HCV) genotype 1b and low virus load.

Methods Inclusion criteria were HCV-genotype 1b, serum HCV RNA level of <100 KIU/mL at the initiation time of treatment. A total of 60 were enrolled in this retrospective cohort study. The treatment period of combination therapy was 39.8±16.1 weeks.

Results Of the 60 study patients, 47 had sustained virological response (SVR) by the intention to treat analysis. SVR occurred when serum HCV RNA was negative 8 weeks after the initiation of the treatment (p=0.004) and continuance of negative HCV RNA during treatment was ≥30 week (p=0.016). In rapid virological response, all of seven patients with continuance of negative HCV RNA 20 to 29 weeks during treatment had SVR. In early virological response nine of 10 patients with continuance of negative HCV RNA of 30 to 39 week during treatment had SVR.

Conclusion The duration of combination therapy for chronic hepatitis C should be determined based on the time of attainment of negative HCV RNA in patients with genotype 1b and low-virus load.

Key words: chronic hepatitis C, peginterferon, ribavirin, HCV genotype 1b, low virus load, duration of treatment

(*Inter Med* 48: 253-258, 2009)

(DOI: 10.2169/internalmedicine.48.1629)

Introduction

Current evidence indicates that combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) is associated with a higher rate of sustained virological response (SVR) compared with interferon (IFN) alone (1-7). Hence, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C patients with high virus-load. Now, the selection of duration of treatment and optimum doses of combination therapy is an area of active investigation (8-16).

However, the dropout rates in patients treated with combi-

nation therapy was higher than those treated with IFN monotherapy (17, 18). On the other hand, some authors have reported that in half of the patients with a low virus load HCV RNA is eradicated by IFN monotherapy. Thus, for patients with a low virus load IFN monotherapy has been recommended as a first choice in Japan. However, there is also controversy over which patients should be treated with what agent and what regimen as a first choice for good prolonged prognosis in chronic hepatitis C patients with a low virus load. There is an ongoing need to refine treatment strategies in patients with a low virus load.

Thus, in the present study, we performed a retrospective study to examine the efficacy of combination therapy in pa-

¹Department of Hepatology, Toranomon Hospital, Tokyo and ²Hepatic Research Unit, Toranomon Hospital, Tokyo

Received for publication August 25, 2008; Accepted for publication October 21, 2008

Correspondence to Dr. Yasuji Arase, es9y-ars@asahi-net.or.jp

tients with genotype 1b and low virus load. Additionally, the relationship between attainment time of negativity of serum HCV RNA after the initiation of combination therapy and the continuance of negative HCV RNA in patients with genotype 1b and low HCV-RNA load of <100 KIU/mL were also evaluated.

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of <100 KIU/mL before treatment; 3) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 4) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 5) leukocytes $>2,000/\text{mm}^3$, platelet count $>80,000/\text{mm}^3$, and bilirubin $<2.0 \text{ mg/mL}$; 6) follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy as well as the potential adverse reactions to each patient and informed consent was obtained from each patient.

From December 2004 to May 2007, 60 HCV patients were enrolled in this retrospective cohort study at the study hospital.

Patients were classified into three groups according to their response to combination therapy: rapid virological response (RVR), defined as undetectable HCV RNA at week 4 after the initiation of combination therapy; early virological response (EVR), defined as undetectable HCV RNA at week 5 to 12 of combination therapy; and late virological response (LVR), defined as undetectable HCV RNA at week 13 to 24 of combination therapy. A SVR was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (19).

Next, predictors of SVR in patients with undetectable HCV RNA in serum during treatment were assessed by the multiple logistic regression analysis. Finally, SVR rate based on the attainment time of negativity of HCV RNA and continuance of negative HCV RNA during combination therapy were examined.

Combination therapy of pegylated-IFN and ribavirin

For the treatment regimen, the peginterferon (Peg-intron, Schering-Plough Pharmaceutical Co., Osaka, Japan) and ribavirin (Rebetol, Schering-Plough) were given at the dose described based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of $1.4 \mu\text{g/kg}$ (range, $1.3\text{-}1.7 \mu\text{g/kg}$) subcutaneously

each week and oral ribavirin at a median dose of 12.0 mg/kg (range, $9.9\text{-}14.9 \text{ mg/kg}$) daily. The peginterferon dose was adjusted according to body weight ($60 \mu\text{g}$ for $\leq 40 \text{ kg}$, $80 \mu\text{g}$ for $>40 \text{ kg}$ and $\leq 60 \text{ kg}$, $100 \mu\text{g}$ for $>60 \text{ kg}$ and $\leq 80 \text{ kg}$, $120 \mu\text{g}$ for $>80 \text{ kg}$ and $\leq 100 \text{ kg}$, and $150 \mu\text{g}$ for $>100 \text{ kg}$). The ribavirin dose was adjusted according to body weight (600 mg for $\leq 60 \text{ kg}$, 800 mg for $>60 \text{ kg}$ and $\leq 80 \text{ kg}$, and $1,000 \text{ mg}$ for $>80 \text{ kg}$). The regimen or treatment period was decided by the physician. A total of 39 patients were treated with a 48-week regimen and 16 patients were given combination therapy for a 24-week regimen. Treatment for the remaining five patients was discontinued because of treatment-related side effects within 26 weeks after the initiation of combination therapy.

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80°C until analyzed. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (20). HCV-genotype was examined by polymerized chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (21). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (19). Clinical evaluation and biochemical and hematological tests were performed at 4 weekly intervals.

Liver histology before IFN therapy

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo), fixed in 10% formalin, and stained with Hematoxylin and Eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were diagnosed according to the system of Desmet et al (22).

Statistical analysis

Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test. Independent factors that might have influenced SVR were studied using multiple logistic regression analysis, and the following variables were evaluated as prognostic factors: sex, age, body mass index, liver staging, a history of interferon therapy, a history of HCV load of $\geq 100 \text{ KIU/mL}$, HCV RNA level, biochemical factors (AST, ALT), platelet count, HCV RNA 4, 8, 12 week after the initiation of IFN therapy, continuous negative period of HCV RNA during IFN therapy and period of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Table 1. Clinical Backgrounds before Combination Therapy of Peginterferon and Ribavirin in Chronic Hepatitis C Patients

	Total	Response			p
		RVR	EVR	LVR	
Patients, n [‡]	60	18	31	6	
Sex, male (%) [‡]	42 (70%)	15 (83%)	23 (74%)	2 (33%)	0.063
Age (yrs) [‡]	51.9±10.1	50.8±9.3	52.1±10.8	53.9±10.9	0.713
BMI [‡]	21.9±3.1	23.2±3.6	21.2±2.9	21.9±2.3	0.177
A history of IFN [‡] , (%)	28 (47%)	7 (39%)	13 (42%)	4 (67%)	0.085
History of maximum HCV RNA level of >100KIU/mL (+/-) [†]	43/17	13/5	21/10	4/2	0.498
HCV RNA(KIU/mL) [§]	52 (<5-99)	43(8-93)	58(<5-99)	72(21-90)	0.498
AST (IU/L) [‡]	58±32	61±47	56±24	51±18	0.480
ALT (IU/L) [‡]	73±52	80 ± 62	69 ± 37	82±59	0.456
FPG(mg/dL) [‡]	93.1±13.6	93.2±13.0	92.5±12.2	97.5±24.6	0.182
Triglyceride (mg/dL) [‡]	92.5±35.2	94.5±27.8	90.6±42.9	93.9±30.2	0.887
Platelet(10 ³ /mm ³) [‡]	18.7±6.3	20.9±4.7	19.6±5.9	13.7±5.6	0.106
Fibrosis staging [†] (Non-LC/LC)	54/6	18/0	26/5	5/1	0.067

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; EVR, early virological response; FPG, fasting plasma glucose; HCV, hepatitis C virus; IFN, interferon; LC, liver cirrhosis; LVR, late virological response; RVR, rapid virological response

Normal reference ranges 6-50 IU/L for ALT, 11-38 IU/L for AST,

[†]Data expressed as number of patients (percentage)

[‡]Data expressed as mean ± standard deviation

[§]Data expressed as median (range)

Result

Clinical characteristics of the patients

A total of 60 patients were enrolled on present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age =52 years, male/female =42/18, and median (range) HCV-RNA=52 (<5-99) KIU/mL. Two of the patients treated with 48-regimen and three out of five discontinued combination therapy due to side effects had positive HCV RNA during combination therapy. Patients with negativity of serum HCV RNA during combination therapy were classified into three groups according to the difference of response: RVR (n=18), EVR (n=31), and LVR (n=6). There were no significant differences in several factors in three groups as shown in Table 1.

Safety and tolerance of IFN

Of the 60 patients included in this study, five discontinued combination therapy because of IFN-related adverse events: one patient each with thrombocytopenia, general fa-

tigue, psychiatric disorder, poor appetite, and cholecystitis. The onset of IFN-related side effects ranged from one to 11 weeks after initiation of IFN therapy. These side effects in five patients disappeared one month after cessation of IFN therapy.

Next, ten of the remaining 55 patients had dose reduction of interferon and/or ribavirin because of side effects: 5 cases of thrombocytopenia, 3 cases of general fatigue, and 2 cases of poor appetite. The onset of dose reduction due to IFN-related side effects ranged from 1 to 26 weeks after initiation of IFN therapy.

Efficacy of treatment

Out of 60 patients enrolled in the present study, 47 patients (78.3%) had SVR by the intention-to-treat analysis. Table 2 shows the differences in the clinical background between patients with SVR and those without SVR. The SVR was significantly associated with the attainment time of negativity of serum HCV RNA and continuance period of negative HCV RNA. Multivariate analysis indicated that non-relapse occurred when serum HCV RNA at week 8 was negative (p=0.004) and continuance of negative HCV RNA during treatment was ≥30 weeks (p=0.016) (Table 3).

Table 2. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR

	SVR (n=47)	Non-SVR (n=13)	p value
Age (years old) †	52.2 ± 10.1	53.4 ± 8.9	0.346
Sex (male/female) †	35/12	8/5	0.488
BMI	21.8 ± 3.2	22.2 ± 3.0	0.732
Liver staging (non-LC /LC)	42/5	12/1	1.00
a history of interferon (+/-)	22/25	6/7	1.00
a history of HCV load of ≥100KIU/mL (+/-)	31/16	7/6	0.520
HCV-load (KIU/mL) *	58 (<5-99)	46 (6-93)	0.375
AST (IU/L) *	49 ± 34	54 ± 22	0.102
ALT (IU/L) *	70 ± 55	83 ± 39	0.082
Platelet (10 ⁴ /mm ³) *	19.0 ± 6.5	17.6 ± 3.8	0.230
HCV RNA (-) 4W	17/46 (37%)	0/10 (0%)	0.023
HCV RNA (-) 8W	35/46 (76%)	1/10 (10%)	0.002
HCV RNA (-) 12W	44/46 (96%)	3/10 (30%)	<0.001
Continuous negative period (week)	34.9 ± 11.6	10.4 ± 12.1	<0.001
Period of IFN therapy (week)	41.6 ± 12.6	28.8 ± 19.6	<0.001

Data are number of patients, median (range) or mean ± standard deviation. p value calculated by the Mann-Whitney U test

*ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virologic response

Table 3. Multivariate Analyses Identifying Predictors of SVR

Factor	Category	Odds ratio	95% Confidence interval	p value
HCV RNA week 8*	+ /-	1/69.1	4.0-1201.4	0.004
Continuance period of negative HCV RNA during treatment (week)	<30 / ≥30	1/34.5	1.9-500.0	0.016

HCV, hepatitis C virus

*HCV RNA at week 8 after the initiation of treatment

SVR based on the attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA

All fifty-five patients with negativity of HCV RNA after the initiation of combination therapy had continuance of negative HCV RNA during combination therapy. SVR rate based on the attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA during combination therapy are shown in Table 4. In the RVR group, all of seven patients with continuance of negative HCV RNA of 20 to 29 week during treatment had SVR. In the EVR group, patients with continuance of 30 to 39 week during treatment had SVR of ≥90%. In the LVR group, patients with continuance of 30 to 39 week during treatment had SVR of 50%.

Discussion

We have described the efficacy of combination therapy of peginterferon and ribavirin in patients infected with HCV genotype 1b and low virus load. The present study was limited to patients with genotype 1 and HCV-load of <100 KIU/mL. Another limitation is that the present study was not a randomized controlled study; thus, the treatment period was varied. Moreover, half of the patients had a history of IFN monotherapy and two-thirds of the patients had a history of maximum HCV RNA level of >100 KIU/mL. Clinical backgrounds of the enrolled patients were varied.

However, several findings from the present study have direct implications for combination therapy for chronic hepatitis C in the future. First, SVR was primarily associated with attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA. The period of combination

Table 4. SVR Based on the Attainment Time of Negative HCV RNA and Continuance Period of Negative HCV RNA during Combination Therapy

Response*	Continuance period of negative HCV RNA (week)					Total
	<10	10-19	20-29	30-39	40-49	
RVR	100% (1/1)	ND	100% (7/7)	ND	100% (10/10)	100% (18/18)
EVR	ND	63% (5/8)	ND	90% (9/10)	100% (13/13)	87% (27/31)
LVR	0% (0/2)	ND	ND	50% (2/4)	ND	33% (2/6)
Total	33% (1/3)	63% (5/8)	100% (7/7)	79% (11/14)	100% (23/23)	85% (47/55)

EVR, early virological response; HCV, hepatitis C virus; LVR, late virological response; ND, not done; RVR, rapid virological response

*Response of HCV RNA means attainment time of negativity of serum HCV RNA after the initiation of combination therapy

therapy is statistically significant by univariate analysis. However, multivariate analysis showed that early undetectable HCV RNA and prolonged negativity of serum HCV RNA during treatment were associated with the SVR. In the RVR group, all seven patients with continuance of negative HCV RNA for 20 to 29 week during treatment had SVR. This result suggests that a short course regimen of 24 or < 24 week in combination therapy may be suitable for patients who have genotype 1, low virus load, and RVR. Earlier studies have reported higher SVR rates in patients with undetectable HCV RNA at week 4 compared to those with detectable HCV RNA (7-9, 23). Jensen et al (8) has reported that patients with RVR should be treated for a short course regimen. On the contrary, it may be necessary to treat patients without RVR with a long course regimen. The present results coincided closely with these earlier results.

Secondly, in the EVR group, patients with continuance of negative HCV RNA of ≥ 30 weeks during treatment had SVR of $\geq 90\%$. However, one-third of the patients with continuance of negative HCV RNA of 10 to 19 weeks relapsed after the termination of therapy. This result suggests that patient with EVR should be given combination therapy for a year. Third, in LVR group, half of the patients with continuance of negative HCV RNA of 30 to 39 weeks during treatment had SVR. This indicates that patients with delayed undetectable HCV RNA should be treated to continue the negativity of serum HCV RNA for a prolonged period of \geq one year to obtain a high rate of SVR.

A previous study (24) indicates that the suitable treatment period of combination therapy for chronic hepatitis C should be determined based on the time of attainment of negative

HCV RNA in patients with genotype 1b and a high virus load of ≥ 100 KIU/mL. Similarly, the present study suggests that in patients with genotype 1b and low-virus load, the period of combination therapy should be determined based on the attainment time of negativity of serum HCV RNA.

It is desirable to expose patients with chronic hepatitis C to the shortest duration of treatment possible to reduce the likelihood of adverse events and minimize costs. Long-term treatment can be associated with serious side effects and is costly. HCV treatment of combination therapy is expensive; a 24-week treatment course costs approximately 20,000 dollars. Thus, the results of this study underscore the importance of changing the duration of treatment based on the difference of attainment time of negative HCV RNA. To attain SVR rate of $\geq 90\%$ in patients with undetectable HCV RNA and continuance of negative HCV RNA during treatment, it is desirable to give a short course regimen of ≤ 20 -29 weeks in the RVR group, 30-39 week in the EVR group. Moreover, in LVR, prolonged combination therapy regimen of >48 weeks may be recommended.

In conclusion, the period of combination therapy for chronic hepatitis C should be determined based on attainment time of negativity of serum HCV RNA and continuance of negative HCV RNA in patients with genotype 1b and low-virus load.

Acknowledgement

The present work was supported in part by grants-in-aid from Okinaka Memorial Institute for Medical Research and the Japanese Ministry of Health, Labour and Welfare. The authors acknowledge the editorial assistance of Thomas Hughes.

References

1. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 358: 958-965, 2001.
2. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 347: 975-982, 2002.
3. Hadziyannis SJ, Sette H, Morgan TR, et al; PEGASYS Interna-