that include patients treated with not only IFN but also PEG-IFN plus RBV, should be performed in the future.

In conclusion, the results of the present study indicated that substitution of aa at position 70 of the HCV-1b core region can predict elevation of serum AFP levels in non-HCC patients, and that eradication of the mutant virus seems to induce normalization of AFP. This finding highlights the importance of eradication of this mutant virus in reducing the risk of hepatocarcinogenesis. 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 wild or mutant type), 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 elevated AFP in non-HCC patients.

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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, Kobayshi 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 genotype1b. 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.

Alpert E, Feller ER. 1978. α-Fetoprotein (AF) in benign liver disease. Gastroenterology 74:856–858.

Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kobayashi M, Kumada H. 2007. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol 79:1095–1102.

Bayati N, Silverman Al, Gordon SC. 1998. Serum alpha-fetoprotein levels and liver histology in patients with chronic hepatitis C. Am J Gastroenterol 93:2452–2456.

Bergstrand CG, Czar B. 1956. Demonstration of a new protein fraction in serum from the human fetus. Scand J Clin Lab Invest 8:174.

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.

Chen TM, Huang PT, Tsai MH, Lin LF, Liu CC, Ho KS, Siauw CP, Chao PL, Tung JN. 2007. Predictors of alpha-fetoprotein elevation in patients with chronic hepatitis C, but not hepatocellular carcinoma, and its normalization after pegylated interferon alfa 2a-ribavirin combination therapy. J Gastroenterol Hepatol 22:669–675.

Chu CW, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, Wu JC, Chang FY, Lee SD. 2001. Clinical, virological, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. J Clin Gastroenterol 32:240–244.

Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC Jr, Perrillo RP, Carey W, Jacobson IM, Payne J, Dienstag JL, et al. 1989. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Group. N Engl J Med 321:1501–1506.

Desmet VJ, Gerber M, Hoofnagle JH, Manna M, Scheuer PJ. 1994. Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 19:1513–1520.

Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, Goodman Z, Banks SM, Hoofnagle JH. 1989. Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. N Engl J Med 321:1506–1510.

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.

Dusheiko GM. 1998. The natural course of chronic hepatitis C: Implications for clinical practice. J Viral Hepatol 9–12.

Elftherious N, Heathcote J, Thomas HC, Sherlock S. 1977. Serum alpha-fetoprotein levels in patients with acute and chronic liver disease. J Clin Pathol 30:704–708.

Hu KQ, Esrailian E, Thompson K, Chase R, Kyulo N, Hassen M, Abdelhalim F, Hillebrand DJ, Runyon BA. 2002. Hepatic steatosis is associated with disease progression of chronic hepatitis C: A large cohort study in the United States. Hepatology 36:349A.

Hu KQ, Kyulo N, Lim N, Elhazin B, Hillebrand DJ, Bock T. 2004. Clinical significance of elevated alpha-fetoprotein (AFP) in patients with chronic hepatitis C, but not hepatocellular carcinoma. Am J Gastroenterol 99:860–865.

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.

Ikeda K, Arase Y, Saitoh S, Kobayashi M, Someya T, Hosaka T, Akuta N, Suzuki Y, Suzuki F, Sezaki H, Kumada H, Tanaka A, Harada H. 2006. Prediction model of hepatocarcinogenesis for patients with hepatitis C virus-related cirrhosis. Validation with internal and external cohorts. J Hepatol 44:1089–1097.

Johnson PJ. 2001. The role of serum alpha-fetoprotein estimation in the diagnosis and management of hepatocellular carcinoma. Clin Liv Dis 5:145–159.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotono 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.

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.

Kew MC, Purves LR, Bersohn I. 1973. Serum alpha-fetoprotein levels in acute viral hepatitis. Gut 14:939–942.

Koike K. 2005. Molecular basis of hepatitis C virus-associated hepatocarcinogenesis: Lessons from animal model studies. Clin Gastroenterol Hepatol 3:S132–S135.

Moriishi K, Okabayashi T, Nakai K, Moriya K, Koike K, Murata S, Chiba S, Tanaka K, Suzuki R, Suzuki T, Miyamura T, Matsuura Y. 2003. Proteasome activator PA28γ-dependent nuclear retention and degradation of hepatitis C virus core protein. J Virol 77:10237–10249.

Moriishi K, Mochizuki R, Moriya K, Miyamoto H, Mori Y, Abe T, Murata S, Tanaka K, Miyamura T, Suzuki T, Koike K, Matsuura Y. 2007. Critical role of PA28γ in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. Proc Natl Acad Sci USA 104:1661–1666.

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.

Niederau 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.

Okamoto K, Akuta N, Kumada H, Kobayashi M, Matsuo Y, Tazawa H. 2007. A nucleotide sequence variation detection system for the core region of hepatitis C virus-1b. J Virol Methods 141:1–6.

Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, Taketa K, Endo Y, Nagataki S. 1993. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 328: 1802–1806.

Silver HK, Gold P, Shuster J, Javitt NB, Freedman SO, Finlayson ND. 1974. Alpha 1-fetoprotein in chronic liver disease. N Engl J Med 291:506–508.

Stein DF, Myaing M. 2002. Normalization of markedly elevated α-fetoprotein in a virologic nonresponder with HCV-related cirrhosis. Dig Dis Sci 47:1686–2690.

Yu ML, Lin SM, Chuang WL, Dai CY, Wang JH, Lu SN, Sheen IS, Chang WY, Lee CM, Liaw YF. 2006. A sustained virological response to interferon or interferon/ribavirin reduces hepatocellular carcinoma and improves survival in chronic hepatitis C: A nationwide, multicentre study in Taiwan. Antivir Ther 11:985–994.

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Efficacy of Low-Dose Intermittent Interferon-Alpha Monotherapy in Patients Infected With Hepatitis C Virus Genotype 1b Who Were Predicted or Failed to Respond to Pegylated Interferon Plus Ribavirin Combination Therapy

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The efficacy of interferon (IFN) monotherapy for non-responders to pegylated interferon (PEG-IFN) plus ribavirin (RBV) combination therapy is still unclear. To evaluate the impact of IFN monotherapy on biochemical response, 200 consecutive patients infected with HCV genotype 1b, who received low-dose intermittent IFN-alpha monotherapy, were investigated. A median IFN dose per day of 3 million units was administered during a median period of 74 weeks. As a whole, the ALT normalization rates were 50.5, 65.9, 58.4, and 61.7% at 4, 12, 24, and 48 weeks, respectively. In 40 patients, who had abnormal AFP levels at the start of treatment, 52.5% achieved normalization of AFP within 48 weeks. Multivariate analysis identified indocyanine green retention rate at 15 min as the parameter that influenced significantly and independently ALT normalization. ALT normalization rates of patients who were predicted to be poor responders to PEG-IFN plus RBV combination therapy (but not substitutions of amino acid 70 and/or 91 in the HCV core region, female sex, and lower levels of low-density lipoprotein cholesterol) were similar to others. Furthermore, the ALT normalization rates in non-responders to combination therapy were 29.2, 60.9, 60.0, and 40.0% at 4, 12, 24, and 48 weeks, respectively. The results suggest that low-dose intermittent IFN monotherapy is an efficacious therapeutic regimen for patients unsuitable for PEG-IFN plus RBV, including non-responders, because it can lead to ALT normalization and thus a reduced risk of hepatocarcinogenesis. ***J. Med. Virol. 80:1363–1369, 2008.***

KEY WORDS: HCV; interferon; ribavirin; ALT; hepatocellular carcinoma; core region; AFP; low-density lipoprotein cholesterol

INTRODUCTION

Hepatitis C virus (HCV) usually causes chronic infection, which 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]. Treatment of HCV-chronic hepatitis with interferon (IFN) can induce viral clearance and marked biochemical and histological improvement [Davis et al., 1989; Di Bisceglie et al., 1989].

Pegylated interferon (PEG-IFN) plus ribavirin (RBV) combination therapy for chronic HCV infection is expensive and associated with severe side effects but treated patients show a high-sustained virological response. Patients who do not achieve sustained virological response need to be identified before the start of combination therapy, in order to avoid unnecessary side effects and high costs. Thus, the safer IFN monotherapy should be selected as the therapeutic regimen for patients unsuitable for PEG-IFN plus RBV therapy. In a series of papers, Akuta et al. [2005a, 2006, 2007a,b,c] studied determinants of the response to PEG-IFN plus RBV in patients with high titers of genotype 1b (≥100 kiloIU [KIU]/ml), which is

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dominant in Japan. They identified substitutions of amino acid (aa) 70 and/or 91 in the HCV core region, female sex, and low levels of low-density lipoprotein cholesterol as independent and significant pretreatment negative predictors associated with virological response. Furthermore, previous studies reported that low-dose intermittent IFN monotherapy, as a treatment strategy, induces biochemical response [i.e., normalization of alanine aminotransferase (ALT) and alpha-fetoprotein (AFP) levels] and reduces the risk of hepatocarcinogenesis, even if patients failed to achieve sustained virological response [Arase et al., 2001, 2007; Nomura et al., 2007; McHutchison et al., 2008]. Hence, low-dose intermittent IFN monotherapy might be beneficial therapeutically in reducing the risk of hepatocarcinogenesis in patients who are predicted to be non-responsive to PEG-IFN plus RBV.

The present study included 200 consecutive patients infected with HCV genotype 1b, who were treated by self-injection of low-dose intermittent natural IFN-alpha. The aims of the study were the following. (1) To investigate the normalization rates of alanine aminotransferase (ALT) and α-fetoprotein (AFP) levels within 48 weeks after the commencement of treatment. (2) To examine the predictive factors associated with ALT normalization. (3) To evaluate the efficacy of IFN monotherapy in patients with predictors of poor response to IFN plus RBV combination therapy. (4) To evaluate the efficacy of IFN monotherapy for non-responders to IFN plus RBV combination therapy.

PATIENTS AND METHODS

Patients

Among 252 consecutive HCV-infected patients who started IFN monotherapy between April 2005 and July 2007 at Toranomon Hospital, 200 were selected in the present study based on the following criteria. (1) Patients treated by self-injection of natural IFN-alpha (Sumiferon®; Sumitomo Pharmaceutical Co., Osaka, Japan). (2) Patients infected with HCV genotype 1b alone. (3) Patients negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo, Japan), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp, Emerville, CA), and positive for HCV RNA qualitative analysis with PCR (Amplicor, Roche Diagnostic Systems, Pleasanton, CA). (4) Patients who have not been treated with antiviral or immunosuppressive agents, except for IFN plus RBV combination therapy, within 6 months of enrolment. (5) Patients free of HCC. (6) Patients free of coinfection with human immunodeficiency virus. (7) Lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake). (8) Patients free of other types of hepatitis, including hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease, and (9) patients who consented to the study.

With regard to the clinical features of 200 patients at the start of IFN monotherapy, there were 103 men and 97 women, aged 27–77 with a median age of 62 years. The median ALT level was 80 IU/L (range, 6–487 IU/L), and the median platelet count was $13.0 \times 10^4/mm^3$ (range, 3.8×10^4–$28.0 \times 10^4/mm^3$). The median viremia level was 1,200 KIU/ml (range, 5–>5,000 KIU/ml) (Table I). Furthermore, 162 of the 200 patients (81%) received IFN-alpha monotherapy by three times per week; the remaining 38 patients (19%) received IFN-alpha monotherapy that included an initial daily administration in the first 8 weeks, followed by three times per week. A median IFN dose per day of 3 million units (MU, range; 3–6 MU) was administered during a median period of 74 weeks (range; 2–118 weeks). Of the 200 patients, 40 had not achieved sustained virological response with prior therapy of IFN plus RBV, and especially 27 patients of them had been treated with adequate combination therapy for at least 24 weeks (median, 43 weeks; range, 24–73 weeks).

Efficient treatment represented normalization of ALT levels (normal reference ranges: 6–50 IU/L) and AFP levels (normal reference ranges: ≤20 μg/L) during and at the end of 48-week treatment protocol.

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital.

Laboratory Investigations

Blood samples were obtained at least once every month from the commencement of treatment, and were tested for ALT and AFP levels. The serum samples were frozen at −80°C within 4 hr of collection and then 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 level was measured quantitatively by PCR (Cobas Amplicor HCV monitor v 2.0 using the 10-fold dilution method, Roche Diagnostics, Indianapolis, IN) at the commencement of treatment. The lower detection limit of the assay was 5 KIU/ml.

Detection of Amino Acid Substitutions in Core Region

With 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 it was compared with the consensus sequence constructed on 50 clinical samples [Akuta et al., 2005a] for detecting substitutions at aa 70 of arginine (wild) or glutamine/histidine (mutant) and aa 91 of leucine (wild) or methionine (mutant). In the present study, aa substitutions of the core region were analyzed by direct sequencing [Akuta et al., 2005a, 2006]. The PCR genotyping could be performed in 193 patients; the remaining seven patients could not be analyzed due to the lack of adequate serum samples obtained before treatment.

Histopathological Examination of the Liver

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman

TABLE I. Patient Profile and Laboratory Data at Commencement of Interferon Monotherapy in 200 Patients Infected With HCV Genotype 1b

Demographic data	
Number of patients	200
Sex (M/F)	103/97
Age (years)*	62 (27–77)
History of blood transfusion	81 (40.5%)
Family history of liver disease	58 (29.0%)
Body mass index (kg/m^2)*	22.8 (15.6–32.9)
Laboratory data*	
Serum aspartate aminotransferase (IU/L)	69 (18–756)
Serum alanine aminotransferase (IU/L)	80 (6–487)
Serum albumin (g/dl)	3.7 (2.6–4.4)
Gamma-glutamyl transpeptidase (IU/L)	49 (11–368)
Leukocyte count ($/mm^3$)	4,000 (1,700–8,100)
Hemoglobin (g/dl)	13.9 (8.9–17.3)
Platelet count ($\times 10^4/mm^3$)	13.0 (3.8–28.0)
Indocyanine green retention rate at 15 min (%)	20 (4–62)
Serum iron (μg/dl)	146 (37–322)
Serum ferritin (μg/L)	136 (<10–1,308)
Creatinine clearance (ml/min)	99 (13–167)
Level of viremia (KIU/ml)	1,200 (5–>5,000)
Alpha-fetoprotein (μg/L)	9 (2–398)
Total cholesterol (mg/dl)	165 (15–296)
High-density lipoprotein cholesterol (mg/dl)	45 (21–80)
Low-density lipoprotein cholesterol (mg/dl)	96 (43–237)
Triglycerides (mg/dl)	93 (46–228)
Uric acid (mg/dl)	5.4 (2.8–9.4)
Fasting blood sugar (mg/dl)	97 (67–228)
Histological findings	
Stage of fibrosis (F1/F2/F3/F4/ND)	45/42/35/19/59
Hepatocyte steatosis (none to mild/moderate to severe/ND)	90/24/86
Treatment	
Interferon dose (million units/day)	3 (3–6)
Presence of initial daily interferon administration	38 (19.0%)
Amino acid substitutions in the core region[a]	
aa 70 (wild/non-wild/ND)	118/72/3
aa 91 (wild/non-wild/ND)	124/69/0
aa 70 and aa 91 (double wild/non-double wild/ND)	76/115/2

Data are number and percentage of patients, except those denoted by *, which represent the median (range) values.
Two patterns of mutant and competitive are indicated as non-wild. The pattern of wild at aa 70 and wild at aa 91 was evaluated as double wild-type, and the other patterns were non-double wild-type. ND, not determined.
[a]Amino acid substitutions were evaluated in 193 patient using pretreatment sera by direct sequencing.

needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), 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 made by an experienced liver pathologist (HK) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on histopathological assessment according to the scoring system of Desmet et al. [1994].

Follow-Up

Clinical and laboratory assessments were performed at least once every month from the commencement of treatment. Adverse effects were monitored clinically by careful interviews and medical examination at least once every month. Patient compliance with treatment was evaluated with a questionnaire. Blood samples were also obtained at least once every month from the commencement of treatment, and were also analyzed for levels of ALT and AFP at various time points. Follow-up time represented the time from the start of treatment until the stop of treatment, or until the last visit.

Statistical Analysis

Analysis of efficacy of treatment was performed on an intention to treat basis. The χ^2 test, Fisher's exact probability test, and Mann–Whitney's *U*-test were used to compare the background characteristics between groups. The cumulative ALT normalization rates were calculated using the Kaplan–Meier technique; differences between the curves were tested using the log-rank test. Statistical analyses of ALT normalization according to groups were calculated using the period from the commencement of IFN monotherapy. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with ALT normalization within 48 weeks after the commencement of treatment. The odds ratios and 95% confidence intervals (95%CI) were also calculated. Potential predictive factors associated with ALT normalization

included the following 29 variables: sex, age, history of blood transfusion, family history of liver disease, body mass index, AST, ALT, albumin, γGTP, leukocyte count, hemoglobin, platelet count, indocyanine green retention rate at 15 min, serum iron, serum ferritin, creatinine clearance, level of viremia, AFP, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, fasting blood sugar, fibrosis stage, hepatocyte steatosis, IFN dose per day, and presence of initial daily IFN administration. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using the SPSS software (SPSS, Inc., Chicago, IL). All P values of less than 0.05 by the two-tailed test were considered significant.

RESULTS

Efficacy of IFN Monotherapy

The rates of ALT normalization in the intention to treat population analysis were evaluated at 0, 4, 12, 24, and 48 weeks after commencement of treatment. As a whole, the rates were 18.0% (36/200), 50.5% (94/186), 65.9% (116/176), 58.4% (90/154), and 61.7% (71/115), respectively. Thus, ALT normalization rates favorably exceeded 50% at 4 weeks. Furthermore, in 40 patients with abnormal AFP levels (≥21 μg/L) at the commencement of treatment, AFP levels of 92.5% (37/40) decreased and those of 7.5% (3/40) increased within 48 weeks. Especially, 52.5% (21/40) achieved normalization of AFP within 48 weeks. These results indicate that low-dose intermittent IFN monotherapy achieved favorable biochemical response.

Predictive Factors Associated With ALT Normalization by Univariate and Multivariate Analysis

The data for the whole population sample were analyzed to determine those factors that could predict ALT normalization within 48 weeks after the commencement of treatment. Univariate analysis identified nine parameters that tended to or significantly correlated with ALT normalization. These included AST ($P = 0.007$), ALT ($P = 0.009$), γGTP ($P = 0.075$), platelets ($P = 0.093$), fibrosis stage ($P = 0.066$), indocyanine green retention rate at 15 min ($P = 0.026$), serum iron ($P = 0.003$), high-density lipoprotein cholesterol ($P = 0.067$), and AFP ($P = 0.046$). These factors were entered into multivariate analysis, which then identified indocyanine green retention rate at 15 min ($P = 0.027$) as the parameter that influenced significantly and independently ALT normalization (Table II).

Efficacy of IFN Monotherapy in Patients With Predictors of Poor Response to PEG-IFN Plus RBV Combination Therapy

Figure 1 shows the prevalence with respect to ALT normalization rates in patients with predictors of poor response to PEG-IFN plus RBV combination therapy.

TABLE II. Factors Associated With ALT Normalization During Interferon Monotherapy, Identified by Univariate and Multivariate Analysis

		Univariate Cox proportional hazard model		Multivariate Cox proportional hazard model	
Factor	Category	Odds ratio (95% CI)	P	Odds ratio (95% CI)	P
Aspartate aminotransferase (IU/L)	1:<70	1		—	—
	2:≥70	0.589 (0.400–0.867)	0.007	—	
Alanine aminotransferase (IU/L)	1:<75	1		—	—
	2:≥75	0.588 (0.395–0.875)	0.009	—	
γ-Glutamyl transpeptidase (IU/L)	1:<50	1		—	—
	2:≥50	0.636 (0.386–1.047)	0.075	—	
Platelets ($\times 10^4/mm^3$)	1:<15.0	1		—	—
	2:≥15.0	1.397 (0.946–2.064)	0.093	—	
Fibrosis stage	1:1.2	1		—	—
	2:3.4	0.627 (0.381–1.031)	0.066	—	
Indocyanine green retention rate at 15 min (%)	1:<20	1		1	—
	2:≥20	0.557 (0.333–0.932)	0.026	0.503 (0.274–0.925)	0.027
Serum iron (μg/dl)	1:<150	1		—	—
	2:≥150	0.522 (0.342–0.797)	0.003	—	
High-density lipoprotein cholesterol (mg/dl)	1:<45	1		—	—
	2:≥45	1.468 (0.973–2.215)	0.067	—	
Alpha-fetoprotein (μg/L)	1:<10	1		—	—
	2:≥10	0.662 (0.441–0.992)	0.046	—	

Only variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate and multivariate Cox proportional hazard model are shown.
95% CI, 95% confidence interval.

According to the substitutions of core aa 70 and aa 91, the ALT normalization rates were 15.7% (18/115) versus 22.4% (17/76) at 0 week, 42.6% (46/108) versus 61.4% (43/70) at 4 weeks, 62.1% (64/103) versus 69.7% (46/66) at 12 weeks, 59.3% (54/91) versus 56.1% (32/57) at 24 weeks, and 58.5% (38/65) versus 63% (29/46) at 48 weeks for non-double wild-type and double wild-type, respectively [not significantly different, except for 4 weeks ($P = 0.021$), Fig. 1A].

According to sex, the ALT normalization rates were 22.7% (22/97) versus 13.6% (14/103) at 0 week, 50.6% (44/87) versus 50.5% (50/99) at 4 weeks, 66.3% (53/80) versus 65.6% (63/96) at 12 weeks, 62.7% (42/67) versus 55.2% (48/87) at 24 weeks, and 67.3% (33/49) versus 57.6% (38/66) at 48 weeks for female and male, respectively (not significant, Fig. 1B).

According to the levels of low-density lipoprotein cholesterol, the ALT normalization rates were 15.5% (9/58) versus 20.0% (23/115) at 0 week, 37.0% (20/54) versus 54.1% (59/109) at 4 weeks, 58.8% (30/51) versus 66% (68/103) at 12 weeks, 58.5% (24/41) versus 53.8% (49/91) at 24 weeks, and 66.7% (22/33) versus 57.4% (39/68) at 48 weeks for low levels (<86 mg/dl) and high levels (≥86 mg/dl), respectively [not significant, except for 4 weeks ($P = 0.047$), Fig. 1C].

The above results indicate that in low-dose intermittent IFN monotherapy, ALT normalization rates of patients with predictors of poor response were not different from those without such predictors.

Efficacy of IFN Monotherapy in Non-Responders to IFN Plus RBV Combination Therapy

The rates of ALT normalization were evaluated in 27 patients who had received prior therapy with adequate IFN plus RBV for at least 24 weeks but showed no sustained virological response. The rates were 14.8% (4/27), 29.2% (7/24), 60.9% (14/23), 60% (12/20), and 40.0% (4/10) at 0, 4, 12, 24, and 48 weeks, respectively. Thus, ALT normalization rates favorably exceeded 60% at 12 weeks. These results indicate that non-responders to IFN plus RBV treated again with low-dose intermittent IFN monotherapy can achieve a favorable biochemical response.

DISCUSSION

For chronic HCV infection, PEG-IFN plus RBV combination therapy is expensive, associated with severe side effects but results in a high-sustained virological response. However, it is desirable to identify those patients who do not achieve sustained virological response before the start of the combination therapy in order to free them of unnecessary side effects and high costs. One alternative option for such patients is IFN

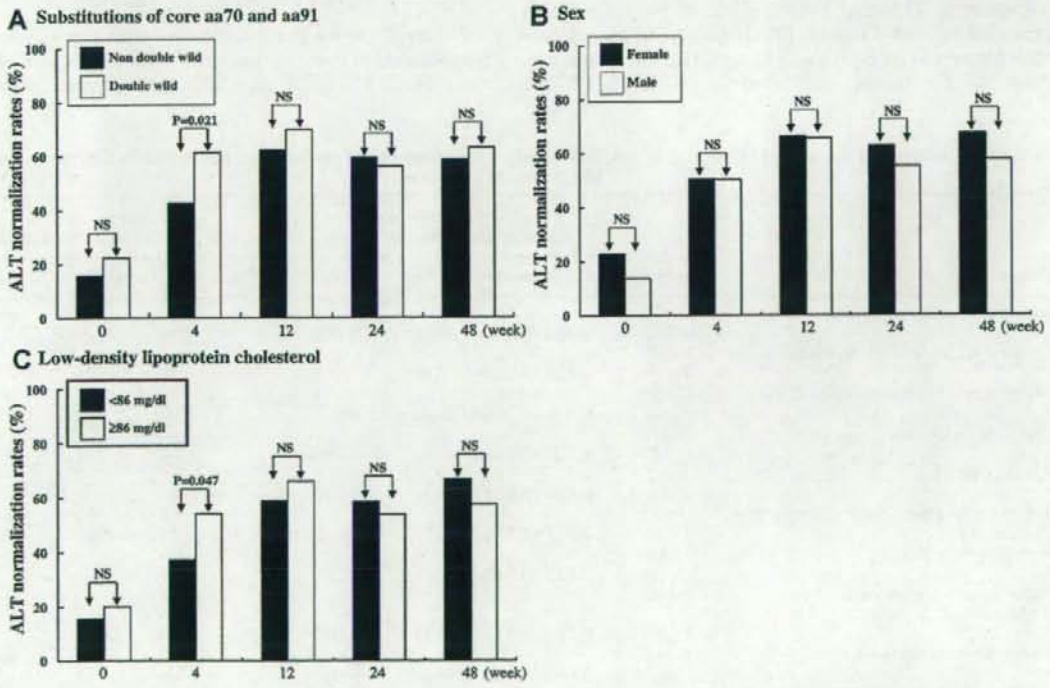

Fig. 1. The ALT normalization rates at 0, 4, 12, 24, and 48 weeks after commencement of IFN monotherapy, according to (A) substitutions of core aa 70 and aa 91, (B) sex, and (C) the levels of low-density lipoprotein cholesterol.

monotherapy, which could reduce the risk of hepatocarcinogenesis [Arase et al., 2001, 2007; Akuta et al., 2005a,b; Donlin et al., 2007; Nomura et al., 2007; McHutchison et al., 2008].

The efficacy of IFN monotherapy in patients who are predicted to respond poorly to IFN plus RBV combination therapy is still unknown. Previous results identified substitutions of aa 70 and/or 91 in the core region, female sex, and lower levels of low-density lipoprotein cholesterol as independent and significant pretreatment negative predictors associated with virological response [Akuta et al., 2005a, 2006, 2007a,b,c]. These studies also showed that substitutions of aa 70 and/or 91 are risk factors for hepatocarcinogenesis in the same patients [Akuta et al., 2007d]. The present study of low-dose intermittent IFN monotherapy showed that ALT normalization rates of patients who were predicted to have a poor response to the combination therapy were not different from others. It is important to achieve a better biochemical response regardless of core aa substitutions as risk factor of hepatocarcinogenesis. Thus, a low-dose intermittent IFN monotherapy for patients predicted to fail to respond to PEG-IFN plus RBV is an efficacious therapeutic regimen for normalization of ALT and thus reduction of risk of hepatocarcinogenesis.

The efficacy of IFN monotherapy for non-responders to IFN plus RBV combination therapy is still controversial. In the present study of low-dose intermittent IFN monotherapy, ALT normalization rates in non-responders to combination therapy favorably exceeded 60% at 12 weeks. Di Bisceglie et al. [2007] reported recently that maintenance therapy with PEG-IFN alpha-2a for non-responder to combination therapy was associated with significant decreases in ALT levels, but the rate of disease progression was similar in treated (34.1%) and untreated (33.8%) groups. The discrepancy between the present results and those of HALT-C trial may be due to one or more factors. The first reason for the difference is probably the large number of cirrhotic patients (about 40% of all) in HALT-C trial. The second reason is probably related to the difference in the efficacy measures used in HALT-C trial, which evaluated decreases in ALT levels regardless of ALT normalization or not. The third reason is probably related to the study design of HALT-C trial with PEG-IFN alpha-2a for non-responders to combination therapy [Di Bisceglie et al., 2007]. Large-scale prospective randomized controlled trials should be conducted in the future to confirm whether low-dose intermittent IFN monotherapy could reduce the risk of hepatocarcinogenesis based on ALT normalization for non-responders to PEG-IFN plus RBV combination therapy.

The present results based on multivariate analysis showed that a high level of indocyanine green retention rate at 15 min is a negative predictor of ALT normalization during the course of low-dose intermittent IFN monotherapy. Previous data indicated that absence of advanced liver fibrosis is a positive predictor of sustained virological response to IFN monotherapy and IFN plus RBV combination therapy [Jouet et al., 1994; Poynard et al., 2000; Bruno et al., 2004]. Akuta et al. [2005a, 2007a] also showed previously that high levels of indocyanine green retention rate at 15 min or low levels of serum albumin are associated with advanced liver fibrosis, and that they are independent and significant negative predictors of the virological response to PEG-IFN plus RBV. To our knowledge, this is the first report that describes the relationship between indocyanine green retention rate at 15 min level and biochemical response during IFN monotherapy. Di Bisceglie et al. [2007] recently reported that maintenance therapy with PEG-IFN alpha-2a failed to halt liver disease progression in patients with advanced hepatic fibrosis. Further studies of large number of patients are required to investigate the relationship between severity of histopathological liver changes and biochemical response during low-dose intermittent IFN monotherapy.

One limitation of the present study was that viral factors other than the core region of HCV genome were not examined, such as the interferon sensitivity determining region (ISDR) and the interferon/ribavirin resistance determining region (IRRDR) of NS5A region [Enomoto et al., 1995, 1996; El-Shamy et al., 2007]. Biochemical response during low-dose intermittent IFN monotherapy seems to be based on a dynamic tripartite interaction of virus, host, and treatment regimen. Further understanding of the complex interaction between these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, the present study showed that one therapeutic regimen that can reduce the risk of hepatocarcinogenesis based on ALT normalization is low-dose intermittent IFN monotherapy, for patients unsuitable for PEG-IFN plus RBV including non-responders. Large-scale prospective randomized controlled trials should be conducted to confirm this finding.

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, Kobayshi M, Arase Y, Ikeda K, Kumada H. 2005a. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Suzuki Y, Sezaki H, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Kumada H. 2005b. Long-term follow-up of interferon monotherapy in 454 consecutive naive patients infected with hepatitis C virus: Multicourse interferon therapy may reduce the risk of hepatocellular carcinoma and increase survival. Scand J Gastroenterol 40:688–696.

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 genotype1b 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.

Arase Y, Chayama K, Ikeda K, Tsubota A, Suzuki Y, Saitoh S, Kobayashi M, Suzuki F, Akuta N, Someya T, Kobayashi M, Kumada H. 2001. Randomized controlled clinical trial of lymphoblastoid interferon-alpha for chronic hepatitis C. Hepatol Res 21:55–66.

Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Kawamura Y, Kobayashi M, Kumada H. 2007. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. J Med Virol 79:1485–1490.

Bruno S, Camma C, Di Marco V, Rumi M, Vinci M, Camozzi M, Rebucci C, Di Bona D, Colombo M, Craxi A, Mondelli MU, Pinzello G. 2004. Peginterferon alfa-2b plus ribavirin for naïve patients with genotype 1 chronic hepatitis C: A randomized controlled trial. J Hepatol 41:474–481.

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.

Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer Jr. HC, Perrillo RP, Carey W, Jacobson IM, Payne J, Dienstag JL, Van Thiel DH, Tamburro C, Lefkowitch J, Albrecht J, Meschievitz C, Ortego TJ, Gibas A. 1989. Treatment of chronic hepatitis C with recombinant interferon alpha. A multicenter randomized, controlled trial. Hepatitis Interventional Group. N Eng J Med 321:1501–1506.

Desmet VJ, Gerber M, Hoofnagle JH, Manna M, Scheuer PJ. 1994. Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 19:1513–1520.

Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, Goodman Z, Banks SM, Hoofnagle JH. 1989. Recombinant interferon alfa therapy for chronic hepatitis C. A randomized double blood, placebo controlled trial. N Eng J Med 321:1506–1510.

Di Bisceglie AM, Shiffman ML, Everson GT, Lindsay KL, Everhart JE, Wright EC, Lee WM, Lok AS, Bonkovsky H, Morgan TR, Dienstag JL, Ghany M, Morishima C, Snow KK. 2007. Prolonged antiviral therapy with peginterferon to prevent complications of advanced liver disease associated with hepatitis C: Results of the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) trial. Hepatology 46:290A.

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.

Dusheiko GM. 1998. The natural course of chronic hepatitis C: Implications for clinical practice. J Viral Hepatol 5(Suppl 1):9–12.

El-Shamy A, Sasayama M, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H. 2007. Prediction of efficient virological response to pegylated interferon/ribavirin combination therapy by NS5A sequences of hepatitis C virus and anti-NS5A antibodies in pre-treatment sera. Microbiol Immunol 51:471–482.

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. 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.

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.

Jouet P, Roudot-Thoraval F, Dhumeaux D, Metreau JM. 1994. Comparative efficacy of interferon alfa in cirrhotic and noncirrhotic patients with non-A, non-B, C hepatitis. Gastroenterology 106: 686–690.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotono 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.

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.

McHutchison JG, Patel K, Schiff ER, Gitlin N, Mur RE, Everson GT, Carithers RL Jr, Davis GL, Marcellin P, Shiffman ML, Harver J, Albercht JK. 2008. Clinical trial: Interferon alfa-2b continuous long-term therapy versus repeated 24-week cycles for retreating chronic hepatitis C. Aliment Pharmacol Ther 27:422–432.

Niederau 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.

Nomura H, Kashiwagi Y, Hirano R, Tanimoto H, Tsutsumi N, Higasgi M, Ishibashi H. 2007. Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: A pilot study. Hepatol Res 37:490–497.

Poynard T, McHutchinson J, Goodman Z, Ling MH, Albrecht J. 2000. Is an "a la carte" combination interferon alfa-2b plus ribavirin regimen possible for the first line treatment in patients with chronic hepatitis C? The ALGOVIRC Group. Hepatology 31:211–218.

The Efficacy of Short-Term Interferon-Beta Therapy for Type C Cirrhotic Patients with Genotype 2a and Low Virus Load

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□ ORIGINAL ARTICLE □

The Efficacy of Short-Term Interferon-Beta Therapy for Type C Cirrhotic Patients with Genotype 2a and Low Virus Load

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Abstract

Objective The aim of this study was to elucidate the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and low virus load.

Methods The present study was retrospective cohort study. Inclusion criteria were liver cirrhosis, hepatitis C virus (HCV) genotype 2a, the serum HCV RNA level of less than 100 KIU/mL, and IFN period of 6 or 8 weeks. Twenty-five consecutive patients who satisfied the above criteria were treated with IFN-beta daily at the dosage of 6 MU for 6 or 8 weeks. Independent factors that might have influenced sustained virologic response (SVR) were studied using multiple logistic regression analysis.

Results Background of clinical profiles were as follows: median (range) age=64 (53-76) years, male/female=13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. Out of 25, 14 patients (56.0%) had SVR by the intention-to-treat analysis. The SVR was significantly associated with serum HCV RNA level. Logistic analysis showed that SVR occurred when HCV RNA level was <50 KIU/mL (p=0.047). Based on the difference of the serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥50 KIU/mL.

Conclusions The 6 or 8-week IFN-beta therapy is a possible selection of therapy for cirrhotic patients with HCV genotype 2a and a serum HCV RNA level of <50 KIU/mL.

Key words: liver cirrhosis, hepatitis C virus, genotype 2a, low virus load, interferon, sustained viral response

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Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies have reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-8). However, IFN is expensive and has a number of serious side effects. Therefore, if the treatment period would become shorter, it could be preferable.

On the other hand, several predictive factors of sustained viral response (SVR) to IFN have been identified, and these include short duration of disease, young age, absence of liver cirrhosis, genotype 2a, low hepatitis C virus (HCV)-RNA levels, HCV and mutant type of nonstructual5A region (9-15). Patients with liver cirrhosis (LC) have a high development of hepatocellular carcinoma (HCC) and progression to decompensated state. Thus, patients with a cirrhotic state should be treated for protection of progression of LC stage. In particular, LC patients with genotype 2a and low HCV-RNA levels might have the possibility of eradication of HCV RNA with a small dose or a short period of interferon (IFN). However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in

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Table 1. Clinical Characteristics before Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load

Characteristics	(n=25)
Age (years old)*	64 (53-76)
Male/female†	13/12
Period of IFN therapy (6w/8w)†	19/6
Total dose of IFN (MU)*	246 (123-336)
HCV load (KIU/mL)*	31 (8-90)
AST (IU/L)*	83 (39-203)
ALT (IU/L)*	74 (27-412)
Hemoglobin (g/dL)*	12.6 (9.7-16.3)
Platelet(10^4/mm^3)*	11.4 (8.0-17.0)
WBC(10^3/mm^3)*	3.8 (3.0-6.9)

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, WBC: white blood cell count.

*Data are expressed as median (range), †Data are number of patients.

LC patients with genotype 2a and low HCV-RNA.

Thus, in this study, we evaluated the efficacy of short-term interferon (IFN) therapy for type C cirrhotic patients with genotype 2a and a low virus load.

Materials and Methods

Patients

A total of 25 consecutive cirrhotic type C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 2002 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated alanine aminotransferase (ALT) elevation of greater than the upper normal limits (ALT normal range: 12-50 IU/L) for more than six months; histological evidence of liver cirrhosis at the time of entry into the trial by the use of distinction equation between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection (16); positive serum HCV RNA; serum HCV RNA level of less than 100 KIU/mL; genotype 2a. We excluded from the study all the patients: 1) with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) Leukocytes <3,000/mm^3, platelets <80,000/mm^3 and bilirubin >1.5 mg/mL before IFN therapy.

Twenty-five patients received IFN at a dose of 6 million units (MU) of natural IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan) daily for 6 or 8 weeks. In general, patients were treated with IFN for 6 weeks and six patients who were treated for 8 weeks were assigned by randomized controlled trial. We regarded sustained virologic response (SVR) to therapy as clearance of HCV RNA by amplicor method (17) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of the clinical trial as well as the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

Blood testing

Blood samples were obtained just before IFN therapy and stored at -80℃. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, USA) (18).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/mL. HCV genotype was examined by the PCR assay, using a mixture of primers for the six sub-

Table 2. Predictive Factors for SVR in Short-term Interferon Therapy in Type C Liver Cirrhosis with Genotype 2a and Low Virus Load

Factor	Category	Odds ratio	95% CI	p value*
HCV RNA (KIU/mL)	<50 /≥50	1/0.09	0.01-0.97	.047
AST (IU/L)	≥76 /<76	1/0.46	0.18-1.17	.102
Age (years)	<60/ ≥60	1/0.22	0.04-1.42	.112
Platelet(10^4/mm^3)	<10/≥10	1/3.00	0.57-15.76	.306
WBC(10^3/mm^3)	<4/≥4	1/2.33	0.46-11.81	.367
Sex	Male /Female	1/0.71	0.14-3.58	.682
ALT (IU/L)	<100/≥100	1/0.75	0.13-4.29	.746
Total dose of IFN (IU/L)	<200/≥200	1/1.29	0.23-7.05	.772
Period of IFN therapy (week)	6/ 8	1/1.19	0.20-6.99	.851

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CI: confidence interval, HCV: hepatitis C virus, IFN: interferon, WBC: white blood cell count.

*p value calculated by logistic regression analysis.

types known to exist in Japan, as reported previously (19).

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, HCV RNA level, liver histology, biochemical factors (AST (aspartate aminotransferase), ALT) before IFN therapy and methods of IFN administration. 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.

Results

Patients' characteristics

Table 1 shows the characteristics of the 25 patients who had performed IFN therapy. Clinical profiles were as follows: median (range) age=64 (53-76) years, male/female= 13/12, and median (range) HCV-RNA=31 (8-90) KIU/mL. All the patients were categorized as Child-Pugh-Turcotte score class A. Of the 25 patients originally included in this study, in five patients the dose of the IFN therapy was reduced from 6 MU to 3 MU because of general fatigue and thrombocytopenia at the time of 1-3 weeks after the initiation of IFN. Thus, the total dose of IFN was 228.0±79.2 million units (MU). The median (range) leukocyte and platelet count in patients with dose reduction were 3.400 (3.100-4.800)/mm^3 and 95.000 (8.8-11.4)/mm^3, respectively, while those in patients without dose reduction were 4.600 (3.000-6.900)/mm^3 and 120.000 (80.000-120.000)/mm^3. Both leukocyte and platelet count in patients without dose reduction were higher than those in patients with dose reduction (leukocyte; p=0.013, platelet; p=0.011).

Efficacy of treatment

Out of twenty-five patients enrolled on present study, 14 patients (56.0%) had SVR by the intention-to-treat analysis.

Table 3. The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR

	SVR	Non-SVR	p value
Age (years old) † (<60/≥60)	7/7	2/9	0.183
Sex (male/female) †	8/6	5/6	0.647
Period of IFN therapy† (6 week/8 week)	9/5	8/3	0.986
Total dose of IFN (MU) † (<200/≥200)	5/9	5/6	0.698
HCV-load (KIU/mL) *	16 (8-69)	66 (23-98)	0.021
AST (IU/L) *	63 (39-203)	85 (53-141)	0.730
ALT (IU/L) *	75 (27-434)	88 (34-230)	0.557
Hemoglobin (g/dL) *	13.7 (10.1-16.3)	11.7 (9.7-16.1)	0.139
Platelet(10^4/mm^3) *	12.2 (8.7-17.0)	10.0 (8.0-16.0)	0.096
WBC(10^3/mm^3) *	4.0 (3.1-6.9)	3.8 (3.0-5.3)	0.841

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HCV: hepatitis C virus, IFN: interferon, MU: million unit, SVR: sustained virologic response, WBC: white blood cell count.

*Data are expressed as median (range), †Data are number of patients,

‡ p value calculated by the Mann-Whitney U test.

The SVR was significantly associated with serum HCV RNA level. The patients with a HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL (Table 2). Based on the difference of serum HCV RNA level, the SVR rate was 68.4% (13/19) in patients with a serum HCV RNA level of <50 KIU/mL and 16.7% (1/6) in patients with a serum HCV RNA level of ≥50 KIU/mL. Table 3 shows the differences in the clinical background between patients with SVR and those without SVR. The serum level of HCV RNA in patients with SVR was lower than that in patients without SVR.

Adverse events

Within one week after the initiation of treatment, flu-like symptoms appeared in all the patients. The leukocyte count was 4,320±1,370/mm^3 and the platelet count was 119,000± 23,000/mm^3 before the initiation of IFN therapy, whereas the values were 2,670±830/mm^3 and 71,000±17,000/mm^3, respectively, two weeks after the initiation of the therapy. None of the patients withdrew from this treatment due to IFN-related side effects.

Discussion

The present study was limited by non-randomized controlled trial. Another limitation of the study was that the number of the patients was small. However, several findings from the present study have direct implications for the short-term IFN treatment of LC patients with genotype 2a and low virus load.

First, more than 50% of patients cleared HCV RNA. This result indicates that the 6- or 8-week regimen of IFN therapy was preferable to eradicate HCV RNA in LC patients with genotype 2a and low virus load. Second, the patients with HCV RNA level of <50 KIU/mL tend to have high SVR compared to those with higher than that in patients with HCV RNA level of ≥50 KIU/mL. On the treatment

period, the efficacy of the 6-week regimen of IFN therapy was almost the same as that of the 8-week regimen. Moreover, the efficacy of the total dose of IFN of <200 MU was not different from that by the total dose of ≥200 MU. These results indicate that in about two-thirds of LC patients with a genotype 2a and serum HCV RNA level of <50 KIU/mL and low virus load, HCV was eradicated by the 6-week regimen or total dose of IFN of <200 MU.

Regarding the side effects of IFN, no patient withdrew the treatment due to IFN-related side effect. Okanoue et al (20) reported that side effects occurred when the daily IFN dose was increased. In the present study, five patients had to reduce the IFN dose due to IFN side effects. On the IFN therapy for LC patients, the physician in charge should check the clinical findings compared to the patients with chronic hepatitis C.

At present, the combined IFN and ribavirin therapy is a standard therapy for chronic hepatitis C patients with genotype 1b and a high load of HCV-RNA. However, prolonged combination therapy of IFN and ribavirin is associated with various side effects. If the total dose of IFN is decreased and the period of IFN therapy is short, it would be desirable from two points of cost and side effect. Fortunately, in patients with low HCV-RNA levels, HCV RNA tends to be eradicated with a small dose of IFN (21-24). The present study indicates that in patients with a low HCV-RNA, HCV RNA can be eradicated with a small dose of IFN.

Conclusion

The present study indicates that the 6 or 8-week of IFN therapy is a possible selection of therapy for liver cirrhotic type C patients with genotype 2a and low virus load.

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References

1. McHutchison JG, Poynard T, Pianko S, et al. The impact of interferon plus ribavirin on response to therapy in black patients with chronic hepatitis C. Gastroenterology **119**: 1317-1323, 2000.
2. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N Engl J Med **339**: 1485-1492, 1998.
3. Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alpha 2b plus ribavirin for 48 weeks or 24 weeks versus interferon alpha 2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet **52**: 1426-1432, 1998.
4. Reichard O, Norkrans G, Fryden A, Braconier JH, Sonnerborg A, Weiland O. Randomised, double-blind, placebo-controlled trial of interferon alpha 2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. Lancet **351**: 83-87, 1998.
5. Schalm SW, Hansen BE, Chemello L, et al. Ribavirin enhances the efficacy but not the adverse effects of interferon in chronic hepatitis C. Meta-analysis of individual patient data from European Centers. J Hepatol **26**: 961-966, 1997.
6. Zeuzem S, Feinman SV, Rasenack J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med **343**: 1666-1672, 2000.
7. Lindsay KL, Trepo C, Heintges T, et al. Hepatitis Interventional Therapy Group. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. Hepatology **34**: 395-403, 2001.
8. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment for chronic hepatitis C: a randomised trial. Lancet **358**: 958-965, 2001.
9. Tsubota A, Chayama K, Ikeda K, et al. Factors predictive of response interferon-alpha therapy in hepatitis C virus infection. Hepatology **19**: 1088-1094, 1994.
10. Di Bisceglie AM, Martin P, Kassianides C, et al. Recombinant interferon alpha therapy for chronic hepatitis C: A randomized, double blind placebo-controlled trial. N Engl J Med **321**: 1506-1510, 1989.
11. Causse X, Godinot H, Chevallier M, et al. Comparison of 1 or 3 MU of interferon alfa-2b and placebo in patients with chronic non-A non-B hepatitis. Gastroenterology **101**: 497-502, 1991.
12. Chayama K, Saitoh S, Arase Y, et al. Effect of interferon administration on serum hepatitis C virus RNA in patients with chronic hepatitis C. Hepatology **13**: 1040-1043, 1991.
13. Reichard O, Glaumann H, Fryden A, et al. Two-year biochemical, virological and histological follow-up in patients with chronic hepatitis C responding in a sustained fashion to interferon alfa-2b treatment. Hepatology **21**: 918-922, 1995.
14. Shiratori Y, Kato N, Yokosuka O, et al. Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Tokyo-Chiba Hepatitis Research Group. Gastroenterology **113**: 558-566, 1997.
15. Enomoto N, Sakuma I, Asahina Y, et al. 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**: 77-811, 996.
16. Ikeda K, Saitoh S, Kobayashi M, et al. Distinction between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection. Practical discriminant function using common laboratory data. Hepatol Res **18**: 252-266, 2000.
17. Albadalejo J, Alonso R, Antinozzi R, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. J Clin Microbiol **36**: 862-865, 1998.
18. Doglio A, Laffont C, Caroli-Bosc FX, Rochet P, Lefebvre J. Second generation of the automated Cobas Amplicor HCV assay improves sensitivity of hepatitis C virus RNA detection and yields results that are more clinically relevant. J Clin Microbiol **37**: 1567-1569, 1999.
19. Dusheiko G, Schmilovitz-Weiss H, Brown D, et al. Hepatitis C virus genotypes; an investigation of type-specific differences in geographic origin and disease. Hepatology **19**: 13-18, 1994.
20. Okanoue T, Sakamoto S, Itoh Y, et al. Side effects of high-dose interferon therapy for chronic hepatitis C. J Hepatol **25**: 283-291, 1996.
21. Dalgard O, Mangia A. Short-term therapy for patients with hepatitis C virus genotype 2 or 3 infection. Drugs **66**: 1807-1815, 2006.
22. Tabaru A, Narita R, Hiura M, Abe S, Otsuki M. Efficacy of short-term interferon therapy for patients infected with hepatitis C virus genotype 2a. Am J Gastroenterol **100**: 862-867, 2005.
23. Sato Y, Tokuue H, Kawamura N, et al. Short-term interferon ther-

apy for chronic hepatitis C patients with low viral load. Hepatogastroenterology **51**: 968-972, 2004.

24. Fujiyama S, Chikazawa H, Honda Y, Tomita K. Effective interferon therapy for chronic hepatitis C patients with low viral loads. Hepatogastroenterology **50**: 817-820, 2003.

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The Efficacy of Short-term Interferon-beta Therapy for Chronic Hepatitis C Patients with Low Virus Load

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□ ORIGINAL ARTICLE □

The Efficacy of Short-term Interferon-beta Therapy for Chronic Hepatitis C Patients with Low Virus Load

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Abstract

Objective The aim of this study was to elucidate the efficacy of short-term interferon (IFN) therapy for chronic hepatitis C patients with low virus load.

Methods The present study was a retrospective cohort study. Inclusion criteria were biopsy-proven chronic hepatitis, the serum hepatitis C virus (HCV) RNA level of less than 100 KIU/ml, IFN period of 8weeks or less. One hundred and eleven consecutive patients satisfied above criteria were treated with IFN-beta (dose: 6 MU, daily for 4, 6, or 8 weeks).

Results Background of clinical profiles were as follows: median (range) age=56 (20-73) years, male/female=64/47, genotype 1b/2a/2b=40/68/3, and median (range) HCV-RNA= 34 (4.5-81) KIU/ml. Out of 111, 64 patients (57.7%) had sustained viral response (SVR). Based on the difference of HCV genotype, the SVR rate was 47.5% (19/40) in genotype 1 and 63.3% (45/71) in genotype 2. In genotype 1, the SVR rate in patients treated with the 8-week-regimen was significantly higher than that in patients treated with the 4- or 6-week regimen. In contrast, in genotype 2, the SVR in patients treated with the 8-week regimen was not significantly different from that in patients treated with the 6-week regimen. None of the patients had severe IFN-related side effects.

Conclusions The 6 or 8-week regiment of IFN-beta therapy is one selection of therapy for chronic hepatitis C patients who have tended to have a SVR and who show IFN-related adverse events.

Key words: chronic hepatitis C, low virus load, interferon, sustained viral response

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Introduction

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies reported improvement of therapeutic efficacy when IFN is combined with ribavirin (1-5). Moreover, novel long-acting formulations of IFN known as pegylated IFN induced higher eradication rate of hepatitis C virus (HCV) (6-8). However, IFN is expensive and has a number of serious side effects. Therefore, if the treatment period becomes shorter, it could be preferable.

Several predictive factors of sustained viral response (SVR) to IFN have been identified, and these include a short duration of disease, young age, absence of liver cirrhosis, low HCV-RNA levels, HCV genotype 2a and mutant type of nonstructural 5A region (9-15). Low dose IFN tends to eradicate HCV RNA in patients who had a low serum level of HCV-RNA. However, there is also controversy over how long the IFN therapy should be continued to eradicate HCV RNA in patients. Thus, in this study we evaluated the duration of IFN therapy in order to eradicate HCV RNA in patients who had low serum levels of HCV-RNA.

Abbreviations: HCV: hepatitis C virus, IFN: interferon, SVR: sustained viral response

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Table 1. Clinical Characteristics before Interferon Therapy in Chronic Hepatitis C Patients*

Characteristics	(n=111)
Age (years old) †	56(20-73)
Male/female ‡	64/47
Liver histology (fibrosis, 1/2/3) ‡	60/25/26
HCV genotype(1b/2a/2b) ‡	40/68/3
HCV load (KIU/ml) †	34 (4.5-81)
AST (IU/L) †	56 (14-226)
ALT (IU/L) †	76 (15-434)

***ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; and HCV, hepatitis C virus.**

† Data are expressed as median(range).

‡ Data are number of patients.

Materials and Methods

Patients

A total of 111 consecutive chronic hepatitis C patients treated with IFN-beta for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan between 1997 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated alanine aminotransferase (ALT) elevation greater than the upper normal limits (ALT normal range: 12-50 IU/L) for more than six months; histological evidence of chronic hepatitis within one year of entry into the trial; positive serum HCVRNA; serum HCV RNA level of less than 100 KIU/ml or 1 Meq/ml; genotype 1b, 2a and 2b. We excluded from the study all of the patients: 1) with concurrent hepatitis B virus (HBV); 2) with a history of IFN therapy; 3) Leukocytes <3,000/mm^3, platelets <80,000/mm^3 and bilirubin >1.5 mg/ml before IFN therapy.

One hundred eleven patients received IFN at a dose of 6 million units (MU) of natural IFN-beta (Toray Industries or Daiichi Pharmaceutical Co., Tokyo, Japan) daily for 4, 6 or 8 weeks. In general, patients were treated with IFN for 8 weeks. Eleven patients treated for 4 weeks and thirty patients treated for 6 weeks were assigned by randomized controlled trial. We regarded sustained viral response (SVR) to therapy as clearance of HCV RNA by RT-nested PCR (16) or amplicor method (17) for more than 6 months after cessation of therapy. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of this clinical trial as well as the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

Blood testing

Blood samples were obtained just before IFN therapy and stored at -80℃. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (18).

On the other hand, serum HCV-RNA at 6 months after the termination of IFN therapy was analyzed by the qualitative PCR assay or RT-nested PCR. The lower detection limit of the qualitative assay is 100 copies/ ml. HCV genotype was examined by the PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously (19).

Liver histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman needle