っていることが明らかとなった。あくまでもMonotherapyのデータであるが、1b型(αIFN:58.9%、βIFN:62.7%)、2a型(αIFN:75.6%、βIFN:83.2%)、2b型(αIFN:64.0%、βIFN:70.7%)であった。さらに我々は、過去にうつ病でIFN治療が完遂しえず、肝炎の再燃をみた症例に対して新たな試みとしてβIFNとリバビリンの併用投与を行った。図1に示すようにαIFNで一旦ウイルスが陰性化したが、うつ病のためIFN治療が充分量行えず、ウイルスは再燃し肝酵素も高値となってきたためβIFN+Riba治療を行い完全著効をえた症例も経験した。このように、治療の工夫をすることでこれまで諦めていた症例をも完全著効に持ち込むことも可能であることが示された。

D. 考　察

IFN治療の目的は完全著効をえることにより肝病態の進展を抑制し、発癌を抑えることにある。近年の飛躍的な治療法の進歩でウイルスを排除する完全著効率はこの15年で著明に向上した。しかしながら、対象患者の高齢化が明らかとなった現在、全ての症例に対して最強の治療と言われているペグインターフェロンとリバビリン併用療法を行うことは副作用中止を考えると不可能と言わざるをえない。このような状況の中で全体の治療効果を上げるためには、新たな工夫が必要と考える。Pilot studyとして症例呈示したように、既存の治療を組み合わせたり、また対象を絞り安全かつ効果的な方法で治療を行うことでこれまで脱落してきた多くの症例をすくいあげることが治療効果の向上につながっていくものと考えている。今後は対象を決めた上でprospectiveに新たな治療を試み、また症例数を積むことでevidenceに基づいた成績を出していきたいと考えている。

E. 結　論

高齢者や合併症を持つ症例においては、治療法を工夫をすることで効果向上が期待しうることが示された。今後は、その治療反応性に関わる要因をさらに解析していくことが肝要である。

F. 健康危険情報

なし

G. 研究発表

1. 論文発表

1) Kawamura Y. et al: The Efficacy of short-term Interferon-beta Therapy for Chronic Hepatitis C Patients with Low Virus Load. Internal Medicine 2008; 47; 355-360

2) Arase Y. et al: Efficacy in Patients with Dose Reduction in Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C. Intervirology 2008; 51; 1-6

3) Arase Y. et al: The Efficacy of 24-Week Interferon Monotherapy for Type C Liver Cirrhosis in Japanese Patients with Genotype 1b and Low Virus Load. Intervirology 2008; 51; 265-269

4) Akuta N. et al: 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. Journal of Medical Virology 2008; 80; 1354-1362

5) Akuta N. et al: 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 Plus Ribavirin Combination Therapy. Journal of Medical Virology 2008; 80; 1363-1369

6) Arase Y. et al: The Efficacy in Short-Term Interferon-Beta Therapy for Type C Cirrhotic with Genotype 2a and Low Virus Load. Internal Medicine 2008; 47; 1085-1090

2. 学会発表

なし

H. 知的財産権の出願・登録状況（予定を含む。）

1. 特許取得：なし

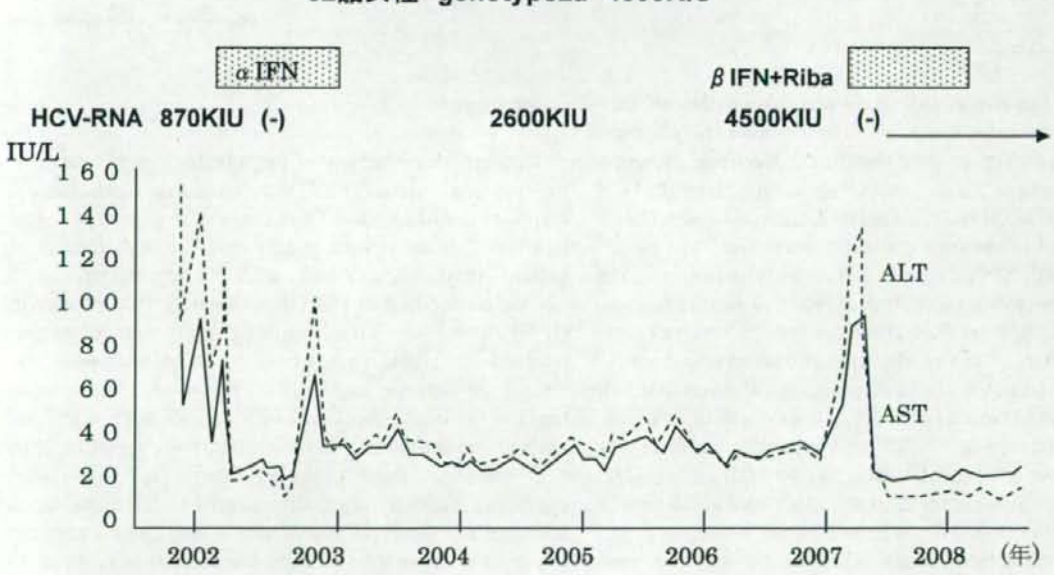
2. 実用新案登録：なし

3. その他：なし

表1. 年齢別にみたIFN24週投与の中止率

	α-IFN	β-IFN	ﾘﾊﾞﾋﾞﾘﾝ併用
60歳未満	129/1775 7.3%	16/414 3.9%	38/349 10.9%
60～64歳	43/379 11.3%	6/192 3.1%	13/86 15.1%
65歳以上	16/140 11.4%	4/73 5.5%	5/19 26.3%

図1. うつの出現のためIFNを中止した症例に対してβIFNを投与し完全著効が得られた症例



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Efficacy in Patients with Dose Reduction in Combination Therapy of Peginterferon and Ribavirin for Chronic Hepatitis C

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Key Words
Chronic hepatitis C · Peginterferon · Ribavirin · Dose reduction

Abstract
Objective: The aim of this study was to elucidate efficacy after dose reduction in combination therapy of peginterferon and ribavirin for chronic hepatitis C. ***Methods:*** Inclusion criteria were hepatitis C virus (HCV) genotype 1b, serum HCV RNA level of ≥100 KIU/ml, dose reduction of peginterferon and/or ribavirin between the first 4 weeks and 20 weeks after the initiation of treatment. 164 patients were enrolled in this retrospective cohort study. Predictive factors for sustained viral response (SVR) after dose reduction were examined. ***Results:*** Out of the 146 patients treated with dose reduction, 57 had SVR. Multivariate analysis showed that SVR occurred when serum HCV RNA at the time of dose reduction was negative ($p < 0.001$) and total ribavirin dose was ≥100% of the anticipated total dose ($p < 0.001$). 57% (55/97) of patients with undetectable serum HCV RNA at the time of dose reduction had SVR. In contrast, only 4% (2/49) of patients with detectable serum HCV RNA at the time of dose reduction had SVR. ***Conclusions:*** On dose reduction of combination therapy for chronic hepatitis C, undetectable serum HCV RNA at the time of dose reduction and attainment of the total ribavirin dose of ≥100% enhance SVR.

Introduction

Combination therapy of peginterferon and ribavirin for hepatitis C virus (HCV) induces sustained virological response (SVR) in 50–60% of cases with genotype 1 and in 80–90% of cases with genotype 2. These SVR rates in patients treated with combination of peginterferon and ribavirin were higher than those treated with interferon (IFN) alone [1–6]. Thus, combination therapy of peginterferon and ribavirin has been recommended as a first choice for chronic hepatitis C. However, combination therapy has been associated with various adverse events, such as psychological disturbances, poor appetite, skin rash, infection, anemia and leukopenia [1, 2, 5, 7]. Dose reduction or even discontinuation of treatment often becomes necessary in combination therapy for chronic hepatitis C. In several studies, the reduction rate due to severe side effects was reported to be about 25–40%.

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Some authors have reported that adherence to combination therapy enhanced sustained virological eradication in genotype 1 with chronic hepatitis C [8–12]. In the present study, we evaluated the efficacy after dose reduction of combination therapy in Japanese patients. The study design is non-randomized retrospective cohort study.

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; (3) dose reduction of peginterferon and ribavirin between the first 4 weeks and 20 weeks after the initiation of combination therapy; (4) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; (5) no hepatitis B surface antigens, antinuclear antibodies, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay, and (6) leukocytes >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/ml. We excluded from the study all the patients with the following: (1) a history of alcohol abuse; (2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The Institutional Ethics Review Board of our hospital approved our study. 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.

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 Pharmaceutical Co.) were given based on body weight. At the initiation of combination therapy, patients received peginterferon at a median dose of 1.5 μg/kg (range 1.2–1.6 μg/kg) subcutaneously each week and oral ribavirin at a median dose of 11.9 mg/kg (range 10.0–16.3 mg/kg) daily. The peginterferon dose was adjusted according to body weight (60 μg for ≤40 kg, 80 μg for >40 and ≤60 kg, 100 μg for >60 and ≤80 kg, and 120 μg for >80 and ≤100 kg). The ribavirin dose was adjusted according to body weight (600 mg for ≤60 kg, 800 mg for >60 and ≤80 kg, and 1,000 mg for >80 kg).

The physician in charge reduced the dose of treatment when the blood cell count decreased. Peginterferon was stepwise reduced 20 μg/week if the WBC declined to <1,500/mm^3, leukocyte count to <750/mm^3 or platelet count to <80,000/mm^3. Ribavirin was stepwise reduced 200 mg if the hemoglobin level declined to ≤10 g/dl. The doses of peginterferon and ribavirin could be increased back to starting doses if these adverse events resolved. The amounts of both medications taken by each patient were expressed as a percentage of the anticipated total dose in the 48-week treatment regimen based on body weight. The median duration of treatment was 48 weeks (range 8–96 weeks). A SVR to therapy was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV, Version 2.0; Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy [13].

Blood samples were obtained just before and 6 months after combination therapy. The samples were stored at –80° until analyzed. Using these blood samples, the HCV RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0; Roche Molecular Systems) [14]. 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 [15]. The start of the follow-up period was defined as the first day of dose reduction in combination therapy. Clinical evaluation and biochemical and hematological tests were performed at 1- to 4-weekly intervals. We evaluated the following: (1) SVR after dose reduction, and (2) predictive factors for SVR after reduction based on combination therapy-related side effects.

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, Japan), 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 scored according to the system of Desmet et al. [16].

Statistical Analysis

A Cox proportional hazards model was used to analyze the factors contributing to the stop of treatment and dose reduction due to combination therapy: factors examined included age, sex, body mass index, histological findings, HCV load, ALT, hemoglobin, WBC, platelet count, HCV RNA at the time of dose reduction, total ribavirin dose and peginterferon dose. Significance of trends in values was determined with a Cochran-Armitage Trend Test. $p < 0.05$ was considered statistically significant. The SPSS software package (SPSS 11.0 for Windows; SPSS Inc., Chicago, Ill., USA) was used for analyses.

Results

Clinical Characteristics of the Patients

A total of 146 patients were enrolled in the present study. The clinical characteristics of patients before combination therapy are shown in table 1. In 15 patients, a liver biopsy was not available because the patients declined to have a biopsy taken. Reduction time after the initiation of combination therapy was 12.0 ± 12.7 weeks (mean ± SD).

Predictors for SVR after Dose Reduction in Combination Therapy

Out of the 146 patients treated with dose reduction, 57 had SVR. Univariate analysis showed that the following seven factors significantly affected the SVR rate in all the patients: HCV RNA at the time of dose reduction ($p < 0.001$), HCV RNA at week 12 ($p < 0.001$), peginterferon

Table 1. Clinical characteristics before combination therapy of peginterferon and ribavirin in chronic hepatitis C patients (n = 146)

Characteristics	Patients, n or median (range)
Age, years	55 (20–69)
Male/female	81/65
Body weight, kg/height, cm	64.9 (36.7–96.6)/ 163.2 (135.2–185.5)
Body mass index	23.1 (16.6–32.0)
History of interferon therapy (–/+)	79/67
Liver histology (fibrosis, mild/moderate/severe)	61/47/23
HCV load, KIU/ml	1,500 (105–5,000)
AST/ALT, IU/l	55 (20–324)/76 (13–580)
Hemoglobin, g/dl	14.4 (10.4–17.9)
Platelets, $\times 10^4/mm^3$	14.1 (8.4–26.0)
WBC, $\times 10^3/mm^3$	4,200 (2,000–8,800)

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; WBC = white blood cells.

and/or ribavirin dose ($p < 0.001$), sex ($p < 0.001$), HCV RNA level before treatment ($p = 0.024$), histopathological staging ($p = 0.031$), and HCV RNA at week 24 ($p = 0.042$) (table 2A). The variables were mutually correlated and multivariate Cox regression analysis was performed with the seven statistically significant variables in the model. As shown in table 2B, multivariate analysis showed that SVR occurred when serum HCV RNA at the time of dose reduction after the initiation of combination therapy was negative ($p < 0.001$) and total ribavirin dose was ≥100% ($p < 0.001$).

SVR Rate Based on Adherence of Combination Therapy in Patients with Dose Reduction

The SVR rate based on adherence of combination therapy in patients with dose reduction was evaluated. Patients were divided into two groups based on the negativity or positivity of HCV RNA at the time of dose reduction after the initiation of combination therapy. Table 3A shows the SVR rate based on adherence of combination

Table 2. Predictive factors for SVR after dose reduction, based on combination therapy-related side effects

A Univariate analysis

Factor	Category	Odds ratio	95% CI	p value
HCV RNA at the time of reduction	–/+	1/0.09	0.04–0.21	<0.001
HCV RNA at week 12	–/+	1/0.09	0.04–0.22	<0.001
Total ribavirin dose, %	<100/≥100	1/8.58	3.99–18.47	<0.001
Total peginterferon dose, %	<100/≥100	1/3.37	1.672–7.04	<0.001
Sex	male/female	1/0.20	0.10–0.42	<0.001
HCV RNA, MEq/ml	<1,000/≥1,000	1/0.43	0.21–0.90	0.024
Liver histology, fibrosis	mild/moderate or severe	1/0.46	0.23–0.93	0.031
HCV RNA at week 24	–/+	1/0.12	0.01–0.92	0.042
Hemoglobin, g/dl	<13/≥13	1/2.29	0.87–5.98	0.092
Platelets, $\times 10^4/mm^3$	<10/≥10	1/2.55	0.86–7.61	0.092
WBC, mm^3	<3,000/≥3,000	1/1.14	0.40–1.27	0.062
ALT, IU/l	≥100/<100	1/1.31	0.59–2.93	0.164
Body mass index	<25/≥25	1/1.24	0.59–2.62	0.566
Age, years	<55/≥55	1/1.13	0.35–3.69	0.838

B Multivariate analysis

Factor	Category	Odds ratio	95% CI	p value
HCV RNA at the time of reduction	–/+	1/0.10	0.04–0.28	<0.001
Total ribavirin dose, %	<100/≥100	1/6.90	2.54–19.05	<0.001

ALT = Alanine aminotransferase; WBC = white blood cells.

Table 3. SVR rate based on adherence of combination therapy in patients with **A** negativity and **B** positivity of serum HCV RNA at the time of dose reduction[1]

A

Total peginterferon dose, %	Total ribavirin dose, %				Total
	<60	61–80	81–100	101–130	
<60	25 (1/4)	40 (2/5)	75 (2/4)	none	38 (5/13)
61–80	0 (0/2)	50 (2/4)	60 (6/10)	100 (2/2)	56 (10/18)
81–100	33 (2/6)	36 (5/14)	50 (7/14)	82 (9/11)	52 (23/44)
101–130	none	none	67 (6/9)	79 (11/14)	77 (17/22)
Total	30 (3/10)	39 (9/23)	57 (21/37)	81 (22/27)	57 (55/97)

B

Peginterferon dose, %	Ribavirin dose, %				Total
	<60	61–80	81–100	101–130	
<60	0 (0/4)	0 (0/2)	0 (0/2)	none	0 (0/8)
61–80	0 (0/2)	0 (0/7)	0 (0/1)	0 (0/4)	0 (0/14)
81–100	0 (0/2)	0 (0/7)	0 (0/2)	20 (1/5)	6 (1/16)
101–130	none	none	0 (0/7)	25 (1/4)	9 (1/11)
Total	0 (0/8)	0 (0/16)	0 (0/12)	15 (2/13)	4 (2/49)

[1] $p = 0.08$ for comparison of the 4 peginterferon groups and $p < 0.001$ for comparison of the 4 ribavirin groups (Cochran-Armitage Trend Test).

therapy in patients with negativity of serum HCV RNA at the time of dose reduction. 55 (57%) of 97 patients with undetectable serum HCV RNA at the time of dose reduction had SVR. A stepwise increase in SVR was observed when the dose of ribavirin was increased ($p < 0.001$, Cochran-Armitage Trend Test). SVR was 42.8% (27/63) in patients who had negativity of serum HCV RNA at the time of dose reduction and had adherence of <100% in both peginterferon and ribavirin. Relapse rate after termination of combination therapy was 43% (42/97) in patients with negativity of serum HCV RNA at the time of dose reduction.

Table 3B shows the SVR rate based on adherence of combination therapy in patients with positivity of serum HCV RNA at the time of dose reduction. Only 2 (2%) of 49 patients with positivity of serum HCV RNA at the time of dose reduction had SVR.

Reasons for Dose Reduction

Of 146 patients with dose reduction, 40 had dose reduction of peginterferon only. 53 patients had dose reduction of ribavirin and 53 patients had both reduction of peginterferon and ribavirin. The cause of reduction accounted for the following: anemia 87 (59.6%), leukopenia 39 (26.7%), thrombocytopenia 22 (15.1%) and other reason, such as general fatigue, 46 (31.5%). 55 of 146 patients with dose reduction had two causes for dose reduction.

Discussion

We have described the efficacy in patients with dose reduction after the initiation of combination therapy of peginterferon and ribavirin for chronic hepatitis C. The present study was limited to patients with genotype 1 and HCV load of ≥100 KIU/ml because previous studies have suggested that SVR in patients with genotype 2 or 3 is not adversely affected by dose reduction [5, 6, 17]. Another limitation of the study was that patients were treated for different durations. This heterogeneity makes it slightly difficult to interpret the results of the study.

However, several findings from the present study have direct implications for dose reduction of the combination therapy of chronic hepatitis C in the future. First, unde-

tectable serum HCV RNA at the time of dose reduction and attainment of total ribavirin dose of ≥100% enhanced SVR in patients with dose reduction. For now, the gold standard of treatment for chronic hepatitis C is a 48-week regimen of combination therapy. Many studies have suggested that reducing the ribavirin dose within the first 12–20 weeks of treatment in patients with genotype 1 was associated with a decline in SVR [8, 9, 18]. The present study indicated that the treatment for >48 weeks and the total ribavirin dose of ≥100% enhanced SVR when patients with a dose reduction showed negativity of serum HCV RNA at the time of dose reduction. Second, most patients with detectable HCV RNA at the time of dose reduction did not have SVR regardless of a peginterferon and ribavirin dose of ≥100%. In patients with detectable HCV RNA at the time of dose reduction, prolonged combination therapy could not enhance SVR.

Several predictive factors of SVR to combination therapy in patients without dose reduction have been identified, and these include amino acid (aa) substitutions in HCV CR (double wild-type; arginine at aa 70/leucine at aa 91), low-density lipoprotein cholesterol (≥86 mg/dl), male gender, γ-glutamyl transpeptidase (<109 IU/l), indocyanine green retention test at 15 min (<10%), and ribavirin dose (≥11.0 mg/kg) [19]. The present study indicates that HCV RNA at the time of dose reduction and total ribavirin dose were good indicators for predicting SVR in patients with dose reduction.

Some studies have suggested that SVR is increased when patients receive a higher dose of peginterferon and/or ribavirin according to body weight [1, 20]. However, in the present study, when the patients had detectable HCV RNA at the time of dose reduction, they had a slight chance of achieving a SVR regardless of prolonged combination therapy with a total dose of ≥100%. Thus, the suitable strategies of combination therapy for chronic hepatitis C patients with dose reduction within the first 20 weeks of treatment are as follows: (1) When the patients show undetectable serum HCV RNA at the time of dose reduction, they should be given combination therapy aimed at SVR; they should be treated with combination therapy of peginterferon and total ribavirin dose of ≥100%. (2) When they show a detectable serum HCV RNA at the time of dose reduction, they should not be given combination therapy aimed at SVR.

In conclusion, on dose reduction of combination therapy in patients with genotype 1b and high virus load, undetectable serum HCV RNA at the time of dose reduction and attainment of the total ribavirin dose of ≥100% enhance SVR.

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References

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

2 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.

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

4 Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM; PEGASYS International Study Group: Peginterferon-alpha-2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–355.

5 Dalgard O, Bjøro K, Hellum KB, Myrvang B, Ritland S, Skaug K, Raknerud N, Bell H: Treatment with pegylated interferon and ribavarin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology 2004;40:1260–1265.

6 Mangia A, Santoro R, Minerva N, Ricci GL, Carretta V, Persico M, Vinelli F, Scotto G, Bacca D, Annese M, Romano M, Zechini F, Sogari F, Spirito F, Andriulli A: Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. N Engl J Med 2005;352:2609–2617.

7 Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M, Shimoe T, Takaguchi K, Hashimoto N, Kobatake T, Tomita M, Kawaguchi M, Kobashi H, Sakaguchi K, Shiratori Y: Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006;43:54–63.

8 McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK; International Hepatitis Interventional Therapy Group: Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061–1069.

9 Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, Lok AS, Morgan TR, Bonkovsky HL, Lee WM, Dienstag JL, Ghany MG, Goodman ZD, Everhart JE; Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis Trial Group: Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. Gastroenterology 2004;26:1015–1023.
10 Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, Bonkovsky HL, Di Bisceglie AM, Lee WM, Dienstag JL, Gretch DR: Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology 2007;132:103–112.
11 Lindahl K, Stahle L, Bruchfeld A, Schvarcz R: High-dose ribavirin in combination with standard dose peginterferon for treatment of patients with chronic hepatitis C. Hepatology 2005;41:275–279.
12 Krawitt EL, Gordon SR, Grace ND, Ashikaga T, Ray MA, Palmer M, Yarze JC, Moskowitz S; for the New York New England Study Team. A study of low-dose peginterferon alpha-2b with ribavirin for the initial treatment of chronic hepatitis C. Am J Gastroenterol 2006;101:1268–1273.
13 Albadalejo J, Alonso R, Antinozzi R, Bogard M, Bourgault AM, Colucci G, Fenner T, Petersen H, Sala E, Vincelette J, Young C: 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 1998;36:862–865.
14 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 1999;37:1567–1569.
15 Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S, McIntyre N, Simmonds P: Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology 1994;19:13–18.
16 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ: Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology 1994;19:1513–1520.
17 Von Wagner M, Huber M, Berg T, Hinrichsen H, Rasenack J, Heintges T, Bergk A, Bernsmeier C, Häussinger D, Herrmann E, Zeuzem S: Peginterferon-alpha-2a (40 kDa) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. Gastroenterology 2005;129:522–527.
18 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J: Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645–652.
19 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410.
20 Bronowicki JP, Ouzan D, Asselah T, Desmorat H, Zarski JP, Foucher J, Bourliere M, Renou C, Tran A, Melin P, Hezode C, Chevalier M, Bouvier-Alias M, Chevaliez S, Montestruc F, Lonjon-Domanec I, Pawlotsky JM: Effect of ribavirin in genotype 1 patients with hepatitis C responding to pegylated interferon alfa-2a plus ribavirin. Gastroenterology 2006;131:1040–1048.

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The Efficacy of 24-Week Interferon Monotherapy for Type C Liver Cirrhosis in Japanese Patients with Genotype 1b and Low Virus Load

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Key Words
Liver cirrhosis · Hepatitis C virus · Genotype 1b · Interferon · Virologic response, sustained

Abstract
Objectives: The aim of this study was to elucidate the efficacy of interferon (IFN) therapy in liver cirrhosis Japanese patients with genotype 1b and low virus load. ***Methods:*** The present study was a retrospective cohort study. Inclusion criteria were liver cirrhosis, HCV genotype 1b, HCV RNA of <100 KIU/ml, and natural IFN-α monotherapy for 24 weeks. In 23 consecutive patients who satisfied the above criteria the efficacy and side effects of IFN were examined. Independent factors that might have influenced a sustained virologic response (SVR) were studied using logistic regression analysis. ***Results:*** The clinical patient profiles were as follows: median (range) age 52 (37–69) years; male/female 18/5, and median (range) HCV RNA 52 (<5–92) KIU/ml. Eight of the 23 patients (34.8%) had SVR by intention-to-treat analysis. Logistic analysis indicated that an HCV RNA level of <50 KIU/ml was associated with SVR ($p = 0.049$). The median (range) HCV RNA levels were 28 (<5–68) KIU/ml in patients with SVR and 60 (6–92) KIU/ml in patients without SVR. ***Conclusions:*** 24-week IFN monotherapy is a suitable therapy for type C cirrhotic patients with genotype 1b and an HCV RNA level of <50 KIU/ml.

Introduction

In patients with chronic hepatitis C, the clearance of serum hepatitis C virus (HCV) RNA has been associated with a good prognosis, including liver histology and liver function improvement and even prolonged survival [1–3]. Interferon (IFN) or combination therapy with IFN and ribavirin is the only evidence-based antiviral therapy widely used for chronic hepatitis C. Recent studies reported that combination therapy with peginterferon and ribavirin was more effective in eradicating HCV compared to IFN monotherapy [4–10]. However, combination therapy with peginterferon and ribavirin has a number of serious side effects compared to IFN monotherapy [11, 12]. Thus, regarding the side effects of IFN therapy, several predictive factors for discontinuing treatment based on IFN

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have been identified, and these include combination therapy with ribavirin, aged patients, reduced blood cell count, and diabetes mellitus complications. On the other hand, clearance of HCV RNA has been reported to be associated with HCV RNA levels, HCV genotype and a mutant type of nonstructural 5A region [13–16].

Patients with liver cirrhosis have a high risk of developing hepatocellular carcinoma (HCC) and progression to a decompensated state. Thus, patients in a liver cirrhotic stage should be treated to protect them from progression to decompensated liver cirrhosis and/or HCC. Although patients with genotype 1 are resistant to IFN therapy, in patients with low HCV RNA levels it might be possible to eradicate HCV RNA with IFN monotherapy even if they are in a liver cirrhotic stage. However, we have no evidence on the optimal schedule of IFN monotherapy for liver cirrhotic patients with genotype 1b and low HCV RNA load. Thus, in this study we tried to elucidate the efficacy of IFN monotherapy in liver cirrhosis patients with genotype 1b and low virus load.

Table 1. Clinical characteristics before 24-week interferon monotherapy in type C liver cirrhosis patients with genotype 1b and low virus load

Characteristics	n = 23
Age, years	52 (37–69)
Male/female	18/5
IFN therapy (2- to 4-week continuous + intermittent/intermittent)	14/9
Total dose of IFN, MU	480 (216–516)
Patients on 3 or 6 MU IFN at initiation	6/17
HCV load, KIU/ml	52 (<5–92)
AST, IU/l	96 (33–348)
ALT, IU/l	101 (33–399)
Hemoglobin, g/dl	14.7 (10.6–16.1)
Platelet count, $\times 10^4/mm^3$	13.4 (8.0–18.8)
WBC count, $\times 10^3/mm^3$	5.2 (3.1–8.5)

Data are expressed either as the median with ranges in parentheses or are the number of patients.

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; HCV = hepatitis C virus; IFN = interferon; MU = million units.

Patients and Methods

A total of 23 consecutive liver cirrhosis type C patients treated with IFN-α for HCV RNA clearance at Toranomon Hospital in Tokyo, Japan, between 2000 and 2006 were enrolled in this study. This study was a retrospective cohort study. Enrollment criteria were: repeated ALT elevation greater than the upper normal limits (ALT normal range 12–50 IU/l) for more than 6 months; laboratory evidence of liver cirrhosis at the time of entry into the trial by the use of a distinction equation between chronic hepatitis and liver cirrhosis in patients with HCV infection [17]; positive serum HCV RNA; genotype 1b, and 24-week treatment with IFN. We excluded from the study all patients with: (1) concurrent hepatitis B virus; (2) a history of IFN therapy, and (3) leukocytes <3,000/mm^3, platelets <80,000/mm^3 and bilirubin >1.5 mg/ml before IFN therapy.

Twenty-three patients received IFN at a dose of 3 or 6 million units (MU) of natural IFN-α (Sumitomo Pharmaceutical Co., Osaka, Japan) for 24 weeks. In 16 patients, daily natural IFN was administered for 2–4 weeks, followed by IFN three times a week for 20–22 weeks. In another 7 patients, natural IFN was administered three times a week for 24 weeks. We regarded a sustained virologic response (SVR) to therapy as clearance of HCV RNA by the Amplicor method [18] 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 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) [19].

On the other hand, serum HCV-RNA 6 months after termination of IFN therapy was analyzed by the qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/ml. The HCV genotype was examined by PCR assay using a mixture of primers for the six subtypes known to exist in Japan as reported previously [20].

Statistical Analysis

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; biochemical factors (AST, ALT); blood cell count before IFN therapy, and methods of IFN administration. The SPSS software package (SPSS Inc., Chicago, Ill., USA) 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 23 patients who received short-term IFN therapy. Clinical profiles were as follows: median (range) age 52 (<5–92) years; male/female = 18/5, and median (range) HCV-RNA 52 (<5–92) KIU/ml. In the 23 patients originally included in

Table 2. Predictive factors for SVR in 24-week interferon therapy in type C liver cirrhosis patients with genotype 1b and low virus load

Factor	Category	Odds ratio	95% CI	p value
HCV RNA, KIU/ml	<50/≥50	1/0.10	0.01–0.99	0.049
AST, IU/l	≥76/<76	1/0.60	0.07–5.06	0.639
Age, years	<60/≥60	1/1.20	0.20–7.18	0.842
Platelet count, $\times 10^4/mm^3$	<10/≥10	1/1.91	0.33–11.01	0.472
WBC count, $\times 10^3/mm^3$	<4/≥4	1/1.11	0.19–6.49	0.907
Sex	male/female	1/0.40	0.04–4.28	0.443
ALT, IU/l	<100/≥100	1/0.59	0.09–3.98	0.590
Total dose of IFN, IU/l	<400/≥400	1/150	0.22–10.30	0.680
Period of IFN therapy, weeks	I/C+I	1/2.63	0.40–17.46	0.318

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; HCV = hepatitis C virus; IFN = interferon; WBC = white blood cell; I/C+I = intermittent/continuous intermittent.

p value calculated by logistic regression analysis.

Table 3. The difference of clinical backgrounds between patients with SVR and those without SVR

	SVR	Non-SVR	p value
Age, years	54 (43–61)	54 (37–69)	0.925
Sex, male/female	7/1	11/4	0.591
Period of IFN therapy, C+I/I	6/2	8/7	0.428
Total dose of IFN, MU (<400/≥400)	3/5	8/7	0.548
HCV load, KIU/ml	28 (<5–78)	60 (6–92)	0.065
AST, IU/l	96 (33–131)	106 (59–348)	0.328
ALT, IU/l	122 (33–171)	144 (26–399)	0.102
Hemoglobin, g/dl	14.7 (13.0–16.1)	14.7 (10.6–16.1)	0.876
Platelet count, $\times 10^4/mm^3$	13.2 (8.0–18.5)	13.6 (9.5–18.8)	0.530
WBC count, $\times 10^3/mm^3$	5.3 (3.2–8.5)	5.2 (3.1–8.3)	0.876

Data are given as either the median with ranges in parentheses or as the number of patients. p value was calculated by the Mann-Whitney U test.

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; HCV = hepatitis C virus; IFN = interferon; MU = million unit; SVR = sustained virologic response; WBC = white blood cell; C+I/I = continuous + intermittent/intermittent.

this study, the median (range) total dose of IFN was 480 (216–524) MU.

Efficacy of Treatment

Of the 23 patients enrolled in the present study, 8 patients (34.8%) had SVR by intention-to-treat analysis. Table 2 shows the predictive factors for SVR during 24-week IFN monotherapy in type C liver cirrhosis patients with genotype 1b and low virus load by using logistic regression analysis. Univariate analysis showed that the HCV RNA level of <50 KIU/ml was significantly associated with the SVR ($p = 0.049$). Table 3 shows the difference in clinical backgrounds between patients with SVR and those without SVR. The median (range) HCV RNA levels were 28 (<5–68) KIU/ml in patients with SVR and 60 (6–92) KIU/ml in patients without SVR.

Safety and Tolerance of IFN

Of the 23 patients included in this study, 1 discontinued IFN therapy because of IFN-related thrombocytopenia. The onset of IFN-related side effects occurred 3 weeks after initiation of IFN therapy. This side effect disappeared 1 month after cessation of IFN therapy.

Next, 3 of 14 patients treated with a dose of 6 MU at the initiation of treatment had a dose reduction to 3 MU because of side effects: 2 cases of thrombocytopenia, and

1 case of general fatigue. The onset of dose reduction due to IFN-related side effects ranged from 2 to 9 weeks after initiation of IFN therapy.

Discussion

This study was performed to elucidate the efficacy of IFN monotherapy in liver cirrhosis patients with genotype 1b and low virus load of <100 KIU/ml. The present study was limited because it was a non-randomized controlled trial. Another limitation to the study was that patients were treated with different doses of IFN (3 or 6 MU) at different frequencies (3 times weekly or daily) for the initiation period. This heterogeneity makes it slightly difficult to interpret the results of the study. However, several findings from the present study have direct implications for IFN treatment of liver cirrhosis patients with genotype 1b and low virus load.

First, in about 30% of the patients treated with IFN monotherapy for 24 weeks, HCV RNA cleared during the 6 months after the termination of IFN therapy. This indicates that the 24-week regimen of IFN therapy could be a suitable therapy to eradicate HCV RNA in liver cirrhosis patients with genotype 1b and low virus load. Second, patients with a HCV RNA level of <50 KIU/ml tend to have high SVR compared to those with a HCV RNA level of ≥50 KIU/ml. Thus, a 24-week regimen of IFN therapy at a dose of 6 MU could be suitable to eradicate HCV RNA in liver cirrhosis patients with genotype 1b and a low virus load of <50 KIU/ml.

Regarding the side effects of IFN monotherapy, 1 patient stopped treatment and 3 patients had to reduce the IFN dose due to IFN-related thrombocytopenia. Okanoue et al. [21] reported that side effects occurred when the daily IFN dose was increased. Moreover, as cirrhotic patients generally have low serum levels of platelets, albumin, and white blood cells, they tend to develop IFN-related side effects. Thus compared to patients with chronic hepatitis C, the physician in charge of liver cirrhosis patients on IFN therapy should rigorously check the clinical findings.

Regarding the development of HCC in 41 liver cirrhosis patients with HCV clearance in our hospital, the cumulative rate of HCC development was 15.6% by the 5th year and 24.2% by the 10th year. On the other hand, the cumulative rate of HCC development in liver cirrhosis patients without HCV clearance was 52–70% by the 10th year [2, 3]. The present study indicates that attainment of SVR in cirrhotic patients reduces the onset of HCC.

At present, combined IFN and ribavirin therapy is standard for chronic hepatitis C patients with a high load of HCV RNA. However, combination therapy with IFN and ribavirin is associated with various side effects. If IFN monotherapy is able to eradicate HCV, it would be desirable from a cost and side effect point of view. Fortunately, in chronic hepatitis C patients with a low virus load, HCV RNA tends to be eradicated with a small dose of IFN monotherapy, even though they have genotype 1 [22, 23].

In conclusion, the present study indicates that 24 weeks of IFN therapy is suitable in cirrhotic patients with HCV genotype 1b and HCV RNA levels of <50 KIU/ml.

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References

1 Hasan F, Jeffers LJ, De Medina M, Reddy KR, Parker T, Schiff ER, Houghton M, Choo QL, Kuo G: Hepatitis C-associated hepatocellular carcinoma. Hepatology 1990;12:589–591.

2 Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H: Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993;328:1797–1801.

3 Ikeda K, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M: A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology 1993;18:47–53.

4 McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK; International Hepatitis Interventional Therapy Group: Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061–1069.

5 Shiffman ML, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, Lok AS, Morgan TR, Bonkovsky HL, Lee WM, Dienstag JL, Ghany MG, Goodman ZD, Everhart JE; Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis Trial Group: Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. Gastroenterology 2004;26:1015–1023.

6 Shiffman ML, Ghany MG, Morgan TR, Wright EC, Everson GT, Lindsay KL, Lok AS, Bonkovsky HL, Di Bisceglie AM, Lee WM, Dienstag JL, Gretch DR: Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology 2007;132:103–112.

7 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.
8 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.
9 Bruno S, Cammà C, Di Marco V, Rumi M, Vinci M, Camozzi M, Rebucci C, Di Bona D, Colombo M, Craxi A, Mondelli MU, Pinzello G: Peginterferon alfa-2b plus ribavirin for naïve patients with genotype 1 chronic hepatitis C: a randomized controlled trial. J Hepatol 2004;41:474–481.
10 Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM; PEGASYS International Study Group: Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346–355.
11 Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M, Shimoe T, Takaguchi K, Hashimoto N, Kobatake T, Tomita M, Kawaguchi M, Kobashi H, Sakaguchi K, Shiratori Y: Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006;43:54–63.
12 Arase Y, Suzuki F, Suzuki Y, Akuta N, Kawamura Y, Kobayashi M, Hosaka T, Sezaki H, Yatsuji H, Kobayashi M, Ikeda K, Kumada H: Side effects of combination therapy of peginterferon and ribavirin for chronic hepatitis-C. Intern Med 2007;46:1827–1832.
13 Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, Hashimoto M, Iwasaki S, Kobayashi M, Hiromitsu K: Factors predictive of response interferon-alpha therapy in hepatitis C virus infection. Hepatology 1994;19:1088–1094.
14 Reichard O, Glaumann H, Fryden A, Norkrans G, Schvarcz R, Sönnerborg A, Yun ZB, Weiland O: 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 1995;21:918–922.
15 Shiratori Y, Kato N, Yokosuka O, Imazeki F, Hashimoto E, Hayashi N, Nakamura A, Asada M, Kuroda H, Tanaka N, Arakawa Y, Omata M: Predictors of the efficacy of interferon therapy in chronic hepatitis C virus infection. Tokyo-Chiba Hepatitis Research Group. Gastroenterology 1997;113:558–566.
16 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.
17 Ikeda K, Saitoh S, Kobayashi M, Suzuki Y, Tsubota A, Suzuki F, Arase Y, Murashima N, Chayama K, Kumada H: Distinction between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection. Practical discriminant function using common laboratory data. Hepatol Res 2000;18:252–266.
18 Albadalejo J, Alonso R, Antinozzi R, Bogard M, Bourgault AM, Colucci G, Fenner T, Petersen H, Sala E, Vincelette J, Young C: 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 1998;36:862–865.
19 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 1999;37:1567–1569.
20 Dusheiko G, Schmilovitz-Weiss H, Brown D, McOmish F, Yap PL, Sherlock S, McIntyre N, Simmonds P: Hepatitis C virus genotypes; an investigation of type-specific differences in geographic origin and disease. Hepatology 1994;19:13–18.
21 Okanoue T, Sakamoto S, Itoh Y, Minami M, Yasui K, Sakamoto M, Nishioji K, Katagishi T, Nakagawa Y, Tada H, Sawa Y, Mizuno M, Kagawa K, Kashima K: Side effects of high-dose interferon therapy for chronic hepatitis C. J Hepatol 1996;25:283–291.
22 Sato Y, Tokuue H, Kawamura N, Nezu-Yajima S, Nakajima H, Ishida H, Takahashi S: Short-term interferon therapy for chronic hepatitis C patients with low viral load. Hepatogastroenterology 2004;51:968–972.
23 Fujiyama S, Chikazawa H, Honda Y, Tomita K: Effective interferon therapy for chronic hepatitis C patients with low viral loads. Hepatogastroenterology 2003;50:817–820.

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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

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Previous studies identified amino acid (aa) substitutions of the hepatitis C virus core region of genotype 1b (HCV-1b core region) and elevated serum alpha-fetoprotein (AFP) levels as predictors of poor virologic response to pegylated interferon (PEG-IFN) plus ribavirin (RBV), and also as risk factors for hepatocarcinogenesis. The present study evaluated the impact of aa substitutions of HCV-1b core region on AFP, as a surrogate marker of hepatocarcinogenesis, on AFP levels in 569 Japanese patients with HCV-1b but without HCC, and investigated the predictive factors of elevated AFP ($\geq$11 μg/L). High AFP levels were detected in 27.4% of the patients. The rate of hepatocarcinogenesis in a group of 109 patients who received IFN monotherapy and followed-up for 15 years, was significantly higher in patients with abnormal than normal AFP. Multivariate analysis of 569 patients identified fibrosis stage (*F*3,4), aspartate aminotransferase ($\geq$76 IU/L), substitution of aa 70 (glutamine or histidine), and platelet count ($<15.0 \times 10^4/\mu l$) as significant determinants of elevated AFP. In 49 patients with abnormal AFP levels and substitutions at aa 70 who were treated with PEG-IFN + RBV, the rate of normalization of AFP was significantly lower in non-virological responders (28.6%) than in transient (71.4%) and sustained (100%) virological responders. The results indicated that substitution of aa 70 of HCV-1b core region is an important predictor of elevated AFP in non-HCC patients, and that eradication of the mutant virus normalizes AFP. The results highlight the importance of eradication of mutant type virus of aa 70 for reducing the risk of hepatocarcinogenesis.
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KEY WORDS: HCV; core region; genotype; AFP; hepatocellular carcinoma; glutamine; histidine

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]. In patients with HCV-chronic hepatitis, treatment with interferon (IFN) can induce viral clearance and marked biochemical and histological improvement [Davis et al., 1989; Di Bisceglie et al., 1989]. Especially, pegylated interferon (PEG-IFN) plus ribavirin (RBV) combination therapy can achieve a high sustained virological response, although patients with non-virological response who remain HCV-RNA-positive at the completion of treatment are also encountered [Akuta et al., 2005, 2006, 2007a,b,c]. Previous studies indicated that amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of genotype 1b (HCV-1b core region) and elevated alpha-fetoprotein (AFP) levels were predictors of poor virological response to PEG-IFN plus RBV therapy [Akuta et al., 2005, 2006, 2007a,b,c; Donlin et al., 2007], and also risk factors and surrogate markers of hepatocarcinogenesis [Ikeda et al., 2006; Akuta et al., 2007d].

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The use of elevated AFP as a predictor of early hepatocarcinogenesis in non-HCC patients might be clinically useful. AFP is a fetal glycoprotein produced by the yolk sac and fetal liver [Bergstrand and Czar, 1956], and has been used widely as a serum marker for the diagnosis of HCC [Sato et al., 1993; Johnson, 2001]. Furthermore, elevated serum AFP is also associated with various chronic liver diseases and hepatic regeneration [Kew et al., 1973; Silver et al., 1974; Elftherious et al., 1977; Alpert and Feller, 1978]. Although a mild rise in serum AFP is commonly seen in chronic HCV-infected patients, its clinicopathological significance remains to be defined. Previous studies indicated that high serum AFP levels correlated with fibrosis stages 3 and 4 [Bayati et al., 1998; Chu et al., 2001; Hu et al., 2002, 2004], levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [Chu et al., 2001; Stein and Myaing, 2002; Hu et al., 2004], prothrombin time [Hu et al., 2004], and HCV-1b [Chu et al., 2001], in chronic HCV-infected patients. However, it is not clear whether mild elevation of AFP in the absence of HCC is associated with eventual development of HCC in HCV-infected patients. Furthermore, the impact of viral factors, such as aa substitutions of HCV-1b core region, on elevated AFP is still unclear.

The aims of the present study conducted in HCC-free Japanese patients infected with HCV-1b, were the following. (1) To evaluate the impact of elevated AFP, especially mild elevation of AFP, on hepatocarcinogenesis in IFN-treated patients without HCC during a long-term (15 years) follow-up period. (2) To identify the impact of aa substitutions in the core region on AFP levels in such patients, and determine the predictive factors for elevated AFP. (3) To investigate the normalization rates of AFP levels after eradication of HCV-RNA by PEG-IFN plus RBV combination therapy.

PATIENTS AND METHODS

Study Population

At Toranomon Hospital, Tokyo, Japan, 2,841 HCV-infected Japanese patients were recruited consecutively into the study protocol of IFN monotherapy between February 1987 and August 2007, and 929 HCV-infected Japanese patients were consecutively recruited into the study protocol of the combination therapy with PEG-IFNα-2b plus RBV between December 2001 and August 2007. Among these, 569 patients were selected in the present retrospective study based on the following criteria. (1) They were negative for hepatitis B surface antigen (radioimmunoassay, Dainabot, Tokyo), positive for anti-HCV (third-generation enzyme immunoassay, Chiron Corp., Emerville, CA), and positive for HCV-RNA qualitative analysis with PCR (nested PCR or Amplicor™, Roche Diagnostics, Indianapolis, IN). (2) They were naive to antiviral treatment. (3) They were infected with HCV-1b alone. (4) AFP levels were measured frequently, and substitutions of aa 70 or 91 in the HCV core region (HCV mutant-70 and HCV mutant-91, respectively) were determined at the commencement of the first course of antiviral treatment. (5) They were free of HCC based on clinical examination, laboratory tests, and imaging studies at baseline. (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 of Toranomon Hospital. Table I summarizes the profiles and laboratory data of the 569 patients at the commencement of antiviral treatment. They included 347 males and 222 females, aged 18–77 years (median, 55 years). Of the total group of 569 patients, 229 received IFN monotherapy, while 340 were treated with PEG-IFN plus RBV combination therapy. Among the patients who received IFN monotherapy, 109 patients started the monotherapy between February 1987 and August 1992, received at least two courses of such therapy, and were followed-up for 15 years. They were evaluated for the rate of development of HCC, associated with a rise in AFP level relative to that measured before the first course IFN monotherapy (baseline). At baseline, the latter group consisted of 80 males and 29 females, aged 22–69 with a median age of 46 years. The numbers of patients with fibrosis stages 1, 2, 3, and 4 were 57, 37, 14, and 1, respectively. The median AST and ALT levels were 85 IU/L (range, 27–400 IU/L) and 138 IU/L (range, 50–594 IU/L), respectively. The median platelet count was $17.0 \times 10^4/\mu l$ (range, 9.8×10^4 to $31.2 \times 10^4/\mu l$). The median viremia level was 5.8 Mequiv./ml (range, <0.5–46.5 Mequiv./ml). The median AFP level was 5 μg/L (range, 2–239 μg/L). The median follow-up time was 16.0 years (range, 0.1–20.3 years). With regard to

TABLE I. Profile and Laboratory Data of 569 Patients Infected with HCV Genotype 1b

Number of patients	569
Sex (male/female)	347/222
Age (years)*	55 (18–77)
Serum aspartate aminotransferase (IU/L)*	59 (17–400)
Serum alanine aminotransferase (IU/L)*	84 (15–594)
Platelet count ($\times 10^4/\mu l$)*	16.1 (3.8–40.2)
Serum alpha-fetoprotein (μg/L)*	6 (2–459)
Fibrosis stage (F1/F2/F3/F4/ND)	227/132/76/17/117
Level of viremia (high titer/low titer)**	522/47
Amino acid substitutions in core region***	
aa 70 (wild/mutant)	340/229
aa 91 (wild/mutant)	341/228
Treatment	
IFN monotherapy/PEG-IFN plus RBV	229/340

Data are number of patients, except those denoted by *, which represent the median (range) values. (**) Level of viremia was evaluated as high titer (≥1.0 Meq/ml, or ≥100 KIU/ml) and low titer (<1.0 Meq/ml, or <100 KIU/ml). (***) The presence of arginine at aa 70 was evaluated as wild type, while other patterns (glutamine/histidine) as mutant type. The presence of leucine at aa 91 was evaluated as wild type, while other patterns (methionine) as mutant type.
Normal reference ranges: 11–38 IU/L for aspartate aminotransferase; 6–50 IU/L for alanine aminotransferase (IU/L); ≤10 μg/L for alpha-fetoprotein. ND: not done; IFN: interferon; PEG-IFN: pegylated interferon; RBV: ribavirin.

the protocol of IFN monotherapy, 68 (62.4%) patients received IFN-α alone; 36 (33.0%) patients received IFN-β alone; while the remaining 5 (4.6%) patients received a combination of IFN-α and IFN-β. The median IFN dose per day of 6 million units (MU, range; 1–10 MU) was administered. IFN monotherapy included initial aggressive induction therapy, consisting of every day within the first 8 weeks of commencement of therapy, followed subsequently by three times per week.

On the other hand, 340 patients received PEG-IFNα-2b combination therapy at a median dose of 1.5 μg/kg (range, 0.8–1.8 μg/kg) subcutaneously each week plus oral RBV at a median dose of 11.0 mg/kg (range, 3.4–14.2 mg/kg) daily for a median duration of 48 weeks (range, 9–112 weeks).

In this study, patients who were HCV-RNA-negative by qualitative PCR analysis at 24 weeks after the completion of therapy, were defined as sustained virological responders. On the other hand, patients who were HCV-RNA-negative by qualitative PCR analysis at the completion of 24-week treatment but became HCV-RNA-positive after the 24-week therapy, were defined as transient virological responders. Patients who remained HCV-RNA-positive by quantitative and/or qualitative PCR analyses at the completion and after treatment, were defined as non-virological responders.

Laboratory Investigations

Blood samples were obtained at least once every month before, during, and after treatment, and were analyzed for AST, ALT, and HCV-RNA 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 nucleotide sequences of NS5 region [Chayama et al., 1993]. HCV-RNA levels were measured by branched DNA assay version 2.0 (Chiron Corp., Emeryville, CA) or quantitative PCR assay (Cobas Amplicor HCV monitor v 2.0 using the 10-fold dilution method, Roche) before, during, and after the antiviral therapy. The lower limits of these assays were 0.5 Meq/ml (10^6 genomic equivalents per milliliter) by branched DNA assay, or 5 KIU/ml by quantitative PCR assay. Samples with undetectable levels by these quantitative assays (<0.5 Meq/ml, or <5 KIU/ml) were checked also by HCV-RNA qualitative analysis with PCR (nested PCR or Amplicor™, Roche) during and after treatment especially, and the results were expressed as positive or negative. The lower limit of the assay was 50 IU/ml. In this study, levels of viremia were evaluated as high titer (≥1.0 Meq/ml, or ≥100 KIU/ml) and low titer (<1.0 Meq/ml, or <100 KIU/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). 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 based on histological assessment according to the scoring system of Desmet et al. [1994].

Detection of Amino Acid Substitutions in Core Region

Okamoto et al. [2007] developed a simple PCR method for detecting substitutions of aa 70 or aa 91 in HCV-1b core region using mutation-specific primer, as an alternative to the direct sequencing method. The major protein type was determined based on the relative intensity of the bands for wild (aa 70: arginine, aa 91: leucine) and mutant HCV-1b (aa 70: glutamine/histidine, aa 91: methionine) in agarose gel electrophoresis. If the intensities of the bands were similar, the case was regarded as competitive. The detection rate was 94.4%, the sensitivity was 10 KIU/ml using quantitative assay with PCR (Cobas Amplicor HCV monitor v 2.0 using the 10-fold dilution method, Roche), the reproducibility was high, and consistency with direct sequencing was 97.1% in positive cases. Mutation in this study refers to substitution from consensus sequence. In previous studies, HCV-J (accession no. D90208) was considered a prototype and the aa substitution was evaluated by comparison with the consensus sequence prepared from 50 clinical trial samples [Kato et al., 1990; Akuta et al., 2005]. In the present study, PCR using primers specific for substitutions of aa 70 or aa 91 was performed in samples collected from 454 patients [Okamoto et al., 2007]; the remaining 115 patients were analyzed by direct sequencing [Akuta et al., 2005, 2006].

Diagnosis of Hepatocellular Carcinoma

Patients were examined for HCC by abdominal ultrasonography every 3–6 months. If HCC was suspected based on ultrasonographic results, additional procedures, such as computed tomography, magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy, were used to confirm the diagnosis.

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. Multiple comparisons were conducted by the Bonferroni test. The cumulative rate of hepatocarcinogenesis was calculated using the Kaplan–Meier technique; differences between carcinogenesis curves between groups were tested using the log-rank test. Statistical analyses of the rate of hepatocarcinogenesis according to

groups were calculated using the period from start of the first course of IFN monotherapy. Univariate and multivariate logistic regression analyses were used to determine the independent predictive factors of elevated AFP. The odds ratios and 95% confidence intervals (95% CI) were also calculated. All P values less than 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 associated with elevated AFP included the following pretreatment variables: sex, age, AST, ALT, platelets, pathological staging, viremia level, and aa substitutions in the core region. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL).

RESULTS

Cumulative Rate of Hepatocarcinogenesis According to AFP Levels

Of the 229 patients who received IFN monotherapy, 109 could be evaluated for the rate of development of HCC based on AFP levels measured at the start of the first course IFN monotherapy (baseline), during a follow-up period of 15 years. All 109 patients received two or more courses of IFN monotherapy; 66 patients received two courses of IFN (including 16 patients who achieved sustained virological response), 35 patients received three courses (including 4 patients who achieved sustained virological response), 7 patients received four courses (including 1 patient who achieved sustained virological response), and one patient received six courses (did not achieve sustained virological response). Thus, 21 of 109 patients achieved sustained virological response after multicourses of IFN monotherapy. For those who received 1, 2, 3, 4, 5, and 6 courses of IFN monotherapy, the median total duration of IFN therapy was 23.9 weeks (range, 0.9–134.7 weeks), 24.0 (range, 1.3–313.7), 25.1 (range, 3.1–193.1), 40.3 (range, 21.0–86.3), 23.6, and 67.9, respectively, and the median total dose of IFN was 526 MU (range, 22–1393 MU), 589 (range, 57–4005), 501 (range, 28–3477), 536 (range, 363–1553), 708, and 1200, respectively. The median cumulative total duration and cumulative total dose, which represented the cumulative total duration and total dose of every course of every patient were 57.7 weeks (range, 14.0–467.6 weeks) and 1380 MU (range, 521–4805 MU), respectively. The median period during which no IFN was administered was 3.7 years (range, 0.1–7.0 years). Finally, the median dose of IFN per week was 22.5 MU (range, 3.7–43.9).

During the follow-up, 8.6% (7 of 81 patients), 20.0% (3 of 15), and 38.5% (5 of 13) developed HCC in patients with AFP levels below 1 (≤10 μg/L), from 1 to 2 (11–20 μg/L), and above twice (≥21 μg/L) the upper limit of normal (ULN), respectively. In patients with AFP levels below 1, from 1 to 2, and above 2 times the ULN, the cumulative hepatocarcinogenesis rates were 0, 7.1, 0% at the end of 5 years; 3.1, 23.4, 37.5% at the end of 10 years; and 14.5, 23.4, 58.3% at the end of 15 years, respectively. The rates were significantly different among the three groups ($P < 0.001$; log-rank test) (Fig. 1). Especially, the rate of hepatocarcinogenesis in patients with normal AFP levels was significantly lower than in those with AFP levels above twice ULN ($P < 0.001$), and tended to be lower than in those with AFP levels from 1 to 2 times ULN ($P = 0.070$). The rate of hepatocarcinogenesis in patients with AFP levels above twice ULN was not significantly higher than in those with AFP levels from 1 to 2 times ULN. Thus, the rate of hepatocarcinogenesis was significantly higher in patients with abnormal AFP levels than in those with normal AFP levels ($P < 0.001$).

Predictive Factors of Elevated AFP in Univariate and Multivariate Analyses

The virological, clinical, and biochemical features of the whole population sample of 569 patients were analyzed to determine factors that could predict elevated AFP (≥11 μg/L). Elevated AFP was detected in 156 of 569 (27.4%) patients. Univariate analysis identified seven parameters that influenced significantly high AFP level. These included age (≥45 years, $P = 0.001$), AST (≥76 IU/L, $P < 0.001$), ALT (≥100 IU/L, $P < 0.001$), platelets ($<15.0 \times 10^4/\mu l$, $P < 0.001$), stage of fibrosis ($F3,4$, $P < 0.001$), and aa substitutions of the core region (mutant type of aa 70, $P < 0.001$, and aa 91, $P = 0.035$). Multivariate analysis identified four parameters that independently influenced high AFP level, including stage of fibrosis ($F3,4$, $P < 0.001$), AST (≥76 IU/L, $P < 0.001$), substitution of aa 70 (mutant type, $P < 0.001$), and platelet count ($<15.0 \times 10^4/\mu l$, $P = 0.019$) (Table IIA).

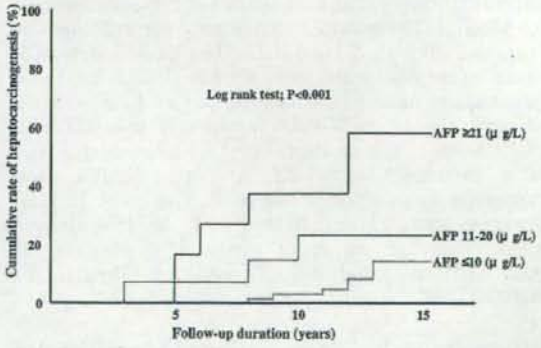

Fig. 1. Cumulative rate of hepatocarcinogenesis according to AFP levels at the start of first course IFN monotherapy. The rate of hepatocarcinogenesis in patients with normal AFP levels (≤10 μg/L) was significantly lower than in those with AFP levels above twice the upper limit of normal (≥21 μg/L) ($P < 0.001$), and tended to be lower than in those with AFP levels from 1 to 2 times the upper limit of normal (11–20 μg/L) ($P = 0.070$). The rate of hepatocarcinogenesis in patients with abnormal AFP levels (≥11 μg/L) was significantly higher than in those with normal AFP levels ($P < 0.001$).

TABLE IIA. Factors Associated with Elevated Serum AFP Levels (≥11 μg/L) in Patients Infected with HCV Genotype 1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Fibrosis stage	1: F1,2	1	
	2: F3,4	5.014 (2.746–9.153)	<0.001
Aspartate aminotransferase (IU/L)	1: <76	1	
	2: ≥76	4.592 (2.707–7.789)	<0.001
Substitution of aa 70	1: wild type	1	
	2: mutant type	2.618 (1.561–4.391)	<0.001
Platelet count ($\times 10^4/\mu l$)	1: ≥15.0	1	
	2: <15.0	1.912 (1.111–3.289)	0.019

*The presence of arginine at aa 70 was evaluated as wild type, while other patterns (glutamine/histidine) as mutant type. Normal reference ranges: ≤10 μg/L for alpha-fetoprotein.

The entire population sample was also analyzed to determine factors that could predict elevated AFP above twice ULN (≥21 μg/L); which was noted in 75 of 569 (13.2%) patients. Univariate analysis identified seven parameters that significantly influenced elevated AFP above twice ULN. These included age (≥45 years, $P = 0.015$), AST (≥76 IU/L, $P < 0.001$), ALT (≥100 IU/L, $P < 0.001$), platelet count ($<15.0 \times 10^4/\mu l$, $P < 0.001$), stage of fibrosis (F3,4, $P < 0.001$), and aa substitutions of the core region (mutant type of aa 70, $P < 0.001$, and aa 91, $P = 0.008$). Multivariate analysis identified four parameters that influenced independently elevated AFP above twice ULN, including stage of fibrosis (F3,4, $P < 0.001$), AST (≥76 IU/L, $P < 0.001$), and aa substitutions of the core region (HCV mutant-91, $P = 0.029$, and -70, $P = 0.056$) (Table IIB).

AFP Levels and aa Substitutions of Core Region

The entire population sample was also analyzed to determine the relationship between aa substitutions of the core region and AFP levels. The proportions of patients with HCV mutant-70 among those with AFP levels below 1, from 1 to 2, from 2 to 4, from 4 to 8, and above 8 times ULN were 33.4% (138 of 413 patients), 53.1% (43 of 81), 60.0% (24 of 40), 66.7% (8 of 12), and 69.6% (16 of 23) (Fig. 2A). Thus, the higher the proportion of patients with HCV mutant-70, the higher the AFP level, and significantly lower proportions of patients with HCV mutant-70 were noted among those with normal AFP levels (33.4%) than those with AFP levels from 1 to 2 times (53.1%) ($P = 0.001$) and above twice ULN (64.0%) ($P < 0.001$).

The proportions of patients with HCV mutant-91 among those with AFP levels below 1, from 1 to 2, from 2 to 4, from 4 to 8, and above 8 times ULN were 37.3% (154 of 413 patients), 40.7% (33 of 81), 67.5% (27 of 40), 25.0% (3 of 12), and 47.8% (11 of 23) (Fig. 2B). Thus, a higher frequency of HCV mutant-91 did not correlate with high AFP levels. In particular, significantly higher proportion of patients with HCV mutant-91 were noted among those with AFP levels from 2 to 4 times ULN (67.5%) than in those with AFP levels below 2 times (37.9%, $P < 0.001$) and above 4 times (40.0%, $P = 0.021$).

Normalization Rates of AFP Levels Based on Eradication of HCV-RNA With PEG-IFN Plus RBV Combination Therapy

Finally, the proportion of patients who showed normalization of AFP after commencement of PEG-IFNα-2b plus RBV combination therapy was determined in those at high risk for hepatocarcinogenesis, who had abnormal AFP levels (>10 IU/L) and HCV mutant-70 at baseline. Of the 340 patients, 49 had both abnormal AFP level and HCV mutant-70 at baseline. Of these, 14.3% (7 of 49 patients) could achieve sustained virological response, 28.6% (14 of 49) showed transient virological response, and 57.1% (28 of 49) had non-virological response. Table III summarizes the characteristics of

TABLE IIB. Factors Associated with Elevated Serum AFP Above Twice the Upper Limit of Normal (≥21 μg/L) in Patients Infected with HCV Genotype 1b, Identified by Multivariate Analysis

Factor	Category	Odds ratio (95% CI)	P
Fibrosis stage	1: F1,2	1	
	2: F3,4	6.875 (3.485–13.56)	<0.001
Aspartate aminotransferase (IU/L)	1: <76	1	
	2: ≥76	6.144 (3.088–12.23)	<0.001
Substitution of aa 91	1: wild type	1	
	2: mutant type	2.101 (1.077–4.099)	0.029
Substitution of aa 70	1: wild type	1	
	2: mutant type	1.914 (0.984–3.722)	0.056

*The presence of arginine at aa 70 was evaluated as wild type, and other patterns (glutamine/histidine) as mutant type. The presence of leucine at aa 91 was evaluated as wild type, and other pattern (methionine) as mutant type. Normal reference ranges: ≤10 μg/L for alpha-fetoprotein.

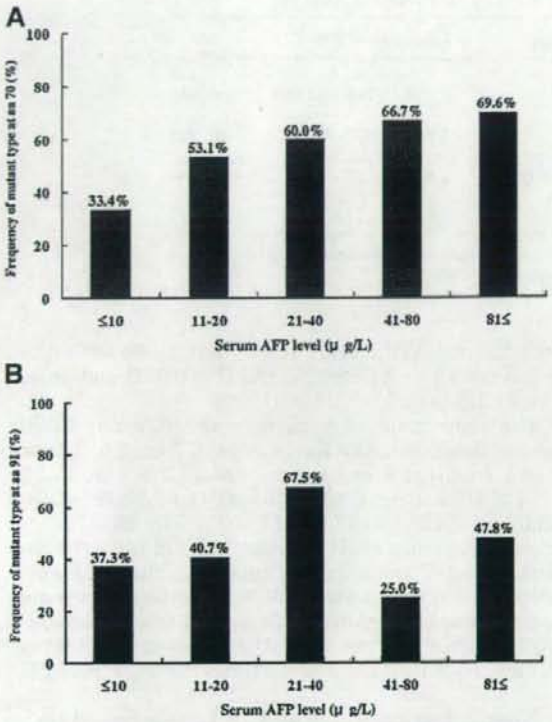

Fig. 2. A: Frequency of mutation in aa at position 70 of the HCV-1b core region according to serum AFP levels. Higher frequencies of the mutation correlated with higher serum AFP levels. Significantly lower frequencies of the mutant type were noted in patients with normal AFP levels (≤10 μg/L) than in those with levels from 1 to 2 times (11–20 μg/L, $P = 0.001$) and above twice the upper limit of normal (≥21 μg/L, $P < 0.001$), respectively. B: Frequency of mutation in aa at position 91 of the HCV-1b core region according to serum AFP levels. Higher frequencies of the mutation did not correlate with higher AFP levels. Significantly higher frequencies of the mutant type were noted in patients with AFP levels from 2 to 4 times the upper limit of normal (21–40 μg/L) than in those with levels below 2 times (≤20 μg/L, $P < 0.001$) and above 4 times (≥41 μg/L, $P = 0.021$).

these 49 patients at the commencement of combination therapy, according to treatment efficacy. The duration of treatment of non-virological responders was significantly shorter than that of sustained- ($P < 0.001$; Bonferroni test) and transient-virological responders ($P = 0.011$; Bonferroni test). Furthermore, AST levels of non-virological responders were significantly lower than those of sustained virological responders ($P = 0.049$; Bonferroni test). However, there were no significant differences in other patient characteristics at the commencement of treatment among the three groups.

The proportions of patients who showed normalization of AFP at the completion of treatment were 71.4% (5 of 7), 71.4% (10 of 14), and 53.6% (15 of 28) for the sustained-, transient-, and non-virological responders, respectively. There were no significant differences in the normalization rates at the completion of treatment among the three groups (Bonferroni test). However, the proportions of patients who showed normalization of AFP at 24 weeks after completion of treatment were 100% (7 of 7), 71.4% (10 of 14), and 28.6% (8 of 28) in the sustained-, transient-, and non-virological responders, respectively. The normalization rate in non-virological responders was significantly lower than in sustained- ($P = 0.001$; Bonferroni test) and transient virological responders ($P = 0.012$; Bonferroni test) (Fig. 3).

DISCUSSION

Elevated AFP in HCV-infected patients without HCC might be useful early predictor of hepatocarcinogenesis, but there is little evidence that mild elevation of AFP in such patients is associated with eventual development of HCC. Ikeda et al. [2006] reported that AFP level above twice ULN was an independent and significant determinant of hepatocarcinogenesis in patients with HCV-related cirrhosis. The present study of HCV-infected patients treated with IFN and followed for up to 15 years also showed that the rate of hepatocarcinogenesis was significantly higher in patients with abnormal AFP levels than in those with normal levels. In particular, the rate of hepatocarcinogenesis in patients with normal AFP levels was significantly lower than in those with levels above twice the ULN, and tended to be lower than in those with levels from 1 to 2 times ULN (i.e., mild elevation of AFP). To our knowledge, the present study is the first to report the hepatocarcinogenesis rates according to AFP levels in HCV-infected patients followed over a 15-year period, including mild elevation of AFP in patients without HCC.

Despite numerous epidemiologic studies linking HCV infection and the development of HCC, it remains controversial whether HCV itself plays direct or indirect role in the pathogenesis of HCC [Koike, 2005]. Studies using transgenic mice concluded that the HCV core region can potentially cause HCC [Moriya et al., 1998], but the clinical impact of HCV core region on hepatocarcinogenesis is still not clear. Previous studies identified substitutions in aa 70 and/or 91 in the HCV-1b core region and elevated AFP levels as predictors of poor virological response to PEG-IFN plus RBV [Akuta et al., 2005, 2006, 2007a,b,c; Donlin et al., 2007], and also as risk factors for hepatocarcinogenesis [Ikeda et al., 2006; Akuta et al., 2007d]. It is speculated that cases resistant to treatment might ultimately develop HCC. The present study indicated that mutation in aa 70 in the core region predicted elevation of AFP in HCV-infected non-HCC patients. These results support the oncogenic potential of the HCV core region and clinically link mutations in this region to HCC.

Previous reports identified PA28γ-dependent pathway as a mechanism of HCV-associated hepatocarcinogenesis. Moriishi et al. demonstrated that knockout of the PA28γ gene induced accumulation of HCV core protein in nuclei of hepatocytes of HCV core gene transgenic mice and disrupted the development of both hepatic steatosis and HCC [Moriishi et al., 2003, 2007]. Furthermore, HCV core protein also enhanced the

TABLE III. Patient Characteristics at Commencement of Combination Therapy of Pegylated Interferon α-2b Plus Ribavirin, of 49 Patients with Abnormal AFP Levels and Mutant Type of aa 70

	SVR (n = 7)	TVR (n = 14)	NVR (n = 28)
Sex (male/female)	3/4	9/5	12/16
Age (years)*	58 (43–64)	56 (34–63)	57 (43–66)
Serum aspartate aminotransferase (IU/L)*	83 (37–324)[a]	84 (34–266)	76 (28–135)
Serum alanine aminotransferase (IU/L)*	99 (41–344)	126 (42–504)	82 (37–218)
Platelet count ($\times 10^4/\mu l$)*	11.6 (8.0–19.3)	14.1 (7.5–20.6)	12.4 (6.6–27.3)
Serum alpha-fetoprotein (μg/L)*	17 (11–161)	21 (11–38)	22 (11–427)
Fibrosis stage (F1/F2/F3/F4/ND)	0/3/2/0/2	2/0/5/0/7	6/3/7/2/10
Level of viremia (high titer/low titer)**	7/0	14/0	27/1
Amino acid substitutions in core region***			
aa 70 (wild/mutant)	0/7	0/14	0/28
aa 91 (wild/mutant)	5/2	6/8	16/12
Treatment duration (weeks)	75 (60–85)[b]	53 (46–77)[c]	47 (12–112)

Data are number of patients, except those denoted by *, which represent the median (range) values. (**) Level of viremia was evaluated as high titer (≥1.0 Meq/ml, or ≥100 KIU/ml) and low titer (<1.0 Meq/ml, or <100 KIU/ml). (***) The presence of arginine at aa 70 was evaluated as wild type, and other patterns (glutamine/histidine) as mutant type.
The presence of leucine at aa 91 was evaluated as wild type, and other pattern (methionine) as mutant type.
Normal reference ranges: 11–38 IU/L for aspartate aminotransferase; 6–50 IU/L for alanine aminotransferase (IU/L); ≤10 μg/L for alpha-fetoprotein.
SVR: sustained virological response; TVR: transient virological response; NVR: non-virological response; ND: not done. [a]$P = 0.049$, [b]$P < 0.001$, [c]$P = 0.011$, compared with NVR by Bonferroni test.

binding of liver X receptor α (LXRα)/retinoid X receptor α (RXRα) to the LXR-response element in the presence of PA28γ [Moriishi et al., 2007]. Thus, PA28γ could play a crucial role in the development of HCV-associated steatogenesis and hepatocarcinogenesis. Further studies are necessary to link the results of animal studies and the clinical impact of aa substitutions in HCV core region on hepatocarcinogenesis.

Chu et al. [2001] indicated that elevation of AFP in the absence of HCC might be associated with HCV-1b infection, and that such rise could correlate with more severe hepatic necroinflammation and fibrosis/cirrhosis and higher viremia levels. The results of the present study indicated that patients infected with HCV mutation-70 had elevated serum AFP levels, although the relation between HCV mutation-91 and AFP was not very clear. On the one hand, multivariate analysis identified HCV mutation-91 as an independent and significant determinant of elevated AFP levels above twice the ULN. On the other; however, a significantly higher proportion of patients infected with HCV mutant-91 had AFP levels from 2 to 4 times ULN compared to those with levels below 2 times and levels above 4 times, i.e., there was no relation between the frequency of HCV mutant-91 and serum AFP levels. Further large-scale studies should be performed to investigate the relationship between HCV mutant-91 and elevated AFP.

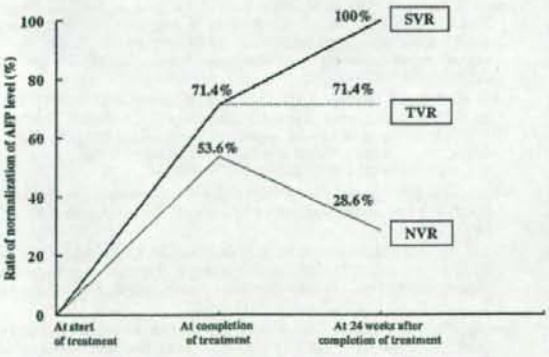

Fig. 3. Normalization rates of AFP levels at and 24 weeks after completion of treatment in sustained virological responders (SVR), transient virological responders (TVR), and non-virological responders (NVR).

Previous studies reported that IFN monotherapy [Arase et al., 2007] and IFN plus RBV combination therapy [Yu et al., 2006; Chen et al., 2007] results in reduction of AFP levels and the likelihood of hepatocarcinogenesis. In the present study, viral eradication (sustained virological response) in patients who received PEG-IFN plus RBV combination therapy was associated with normalization of AFP in patients at high risk for hepatocarcinogenesis (i.e., those with abnormal AFP levels and HCV mutant-70). These results emphasize that the risk of hepatocarcinogenesis could be reduced by eradication of HCV mutant-70. The results also showed that the proportion of patients with normalization of AFP levels was significantly higher in transient virological responders than in non-virological responders, suggesting that transient virological response could also result in the suppression of hepatocarcinogenesis, even when a sustained virological response is not achieved. In Japan, only 3 years had elapsed since the introduction of PEG-IFNα-2b plus RBV combination therapy into the Japanese Government Health Insurance system, and accordingly, the long-term effects of this combination therapy on hepatocarcinogenesis could not be evaluated in the present study. Further studies