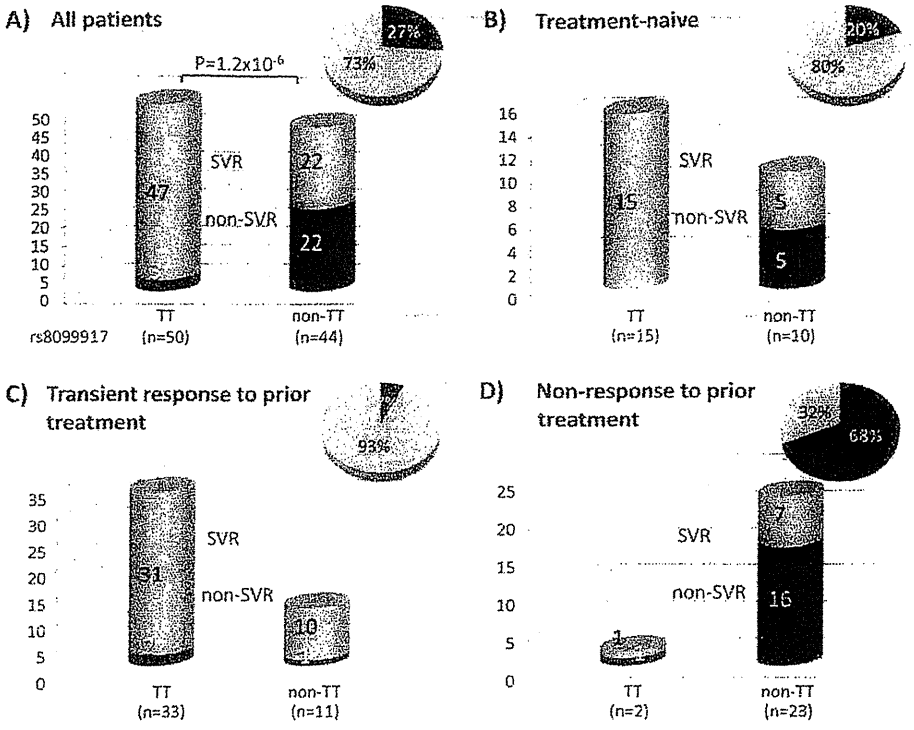

Figure 1. SVR frequency after triple therapy grouped by IL28B SNP rs8099917 genotype and by response to previous interferon (IFN) treatment. *A*, All patients. *B*, Treatment-naive patients. *C*, Previously treated patients who responded transiently to therapy. *D*, Previously treated patients who failed to respond to therapy. Inset pie charts indicate percentage of SVR *(light gray)* and non-SVR *(dark gray)* patients.

resulted in dose reduction according to the treatment protocol, no significant effects on SVR rate resulting from dose reduction were observed.

Viral Substitutions

The 43 patients (46%) with a substitution at position 70 of the HCV core protein (core70) were significantly less likely to achieve SVR than were patients with wild-type core70 (60% vs 86%; $P = .01$). There was no difference in SVR rate due to substitution at position 91 (core91; 81% vs 67%; $P = .17$) (Figure 2). There was also no difference in SVR rate due to substitutions in the NS5A ISDR region ($P = .43$). Patients with rs8099917 genotype TT were significantly more likely to be associated with wild-type core70 or core91 ($P = .006$ and $P = .031$, respectively). There was no association between rs809917 genotype and ISDR substitutions ($P = .94$).

Predictive Factors for RVR

RVR, defined as undetectable HCV RNA levels at week 4 of treatment, is a strong on-treatment predictor of SVR [34]. Previous IFN treatment, time to first ribavirin dose reduction, and baseline hemoglobin levels were each significant univariate predictors, but only hemoglobin level was a significant independent predictor of RVR under multiple logistic regression ($P = .028$; OR, 3.11).

Predictive Factors for SVR

Significant univariate predictors for SVR included clinical factors (γGTP level; rs8099917 genotype), viral factors (core70 substitutions), response to prior treatment (relapse or nonresponse), and on-treatment factors (RVR) (Table 3). Of these, nonresponse to prior treatment, rs8099917 genotype, RVR, and core70 substitutions were retained in the multivariate model, and nonresponse to prior treatment (OR, .17; $P = .01$), rs8099917 genotype (OR, .12; $P = .014$), and RVR (OR, 14.0; $P = .0064$) were identified as significant independent predictors for SVR. When only pretreatment factors were considered, nonresponse to prior treatment (OR, .14; $P = .0028$) and rs8099917 genotype (OR, .19; $P = .027$) were the only independent predictors.

DISCUSSION

This study showed that patients undergoing PEG-IFN, ribavirin, and telaprevir triple therapy for chronic hepatitis C genotype 1 infection achieve a higher SVR rate than typically expected under combination therapy alone in Japanese patients. Moreover, patients who showed transient response in previous treatment were more likely to achieve SVR after triple therapy, whereas nonresponders to prior treatment remained unlikely to eradicate the virus. Considering that telaprevir has a mode of

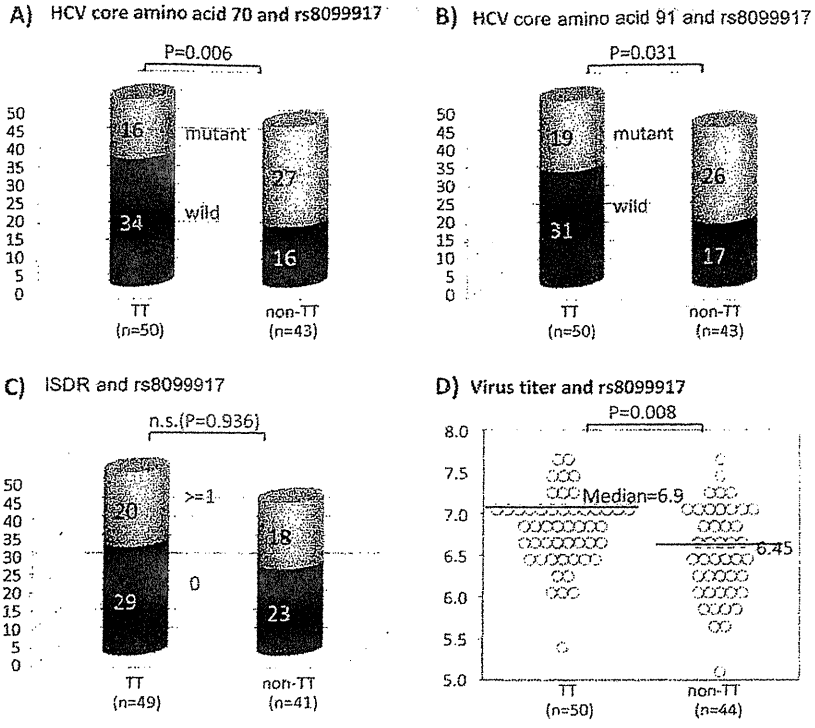

Figure 2. Viral factors and IL128B SNP rs8099917 genotype. *A*, Substitutions at HCV core amino acid 70. *B*, Substitutions at core amino acid 91. *C*, Frequency of patients with ≥2 substitutions in the NS5A interferon sensitivity determining region. *D*, Baseline viral load.

action different from that of IFN and ribavirin, [5] it is surprising that triple therapy does not better improve SVR rates among prior nonresponders, suggesting that additional unknown factors contribute to nonresponse. However, the duration of triple therapy, followed by standard of care, was limited to 24 weeks in this study; therefore, it is possible that prior nonresponders and patients who experienced relapse may benefit from a longer duration of therapy.

The most interesting result from this study is the high SVR rate among patients who previously experienced relapse, even

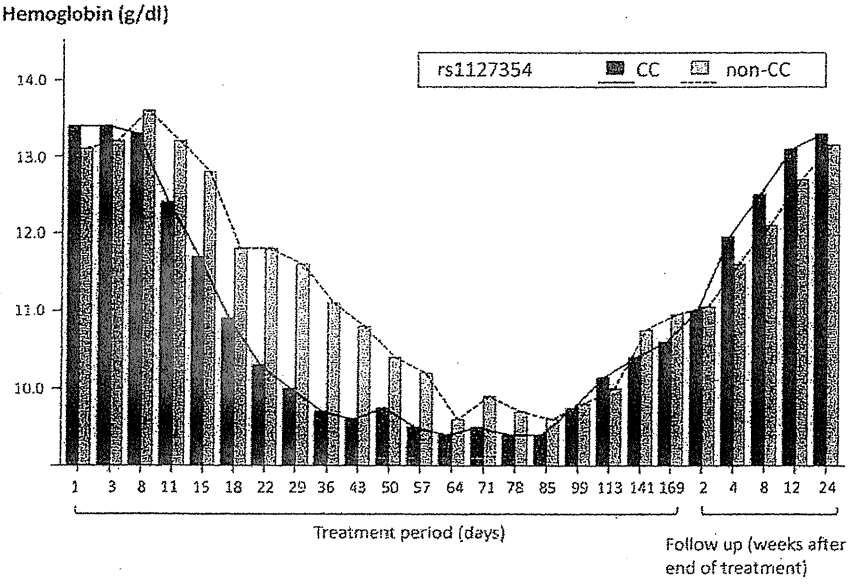

Figure 3. Change in hemoglobin level by ITPA SNP during triple therapy. Hemoglobin levels in patients grouped by ITPA SNP rs1127354 genotype (solid line represents CC; dashed line represents non-CC).

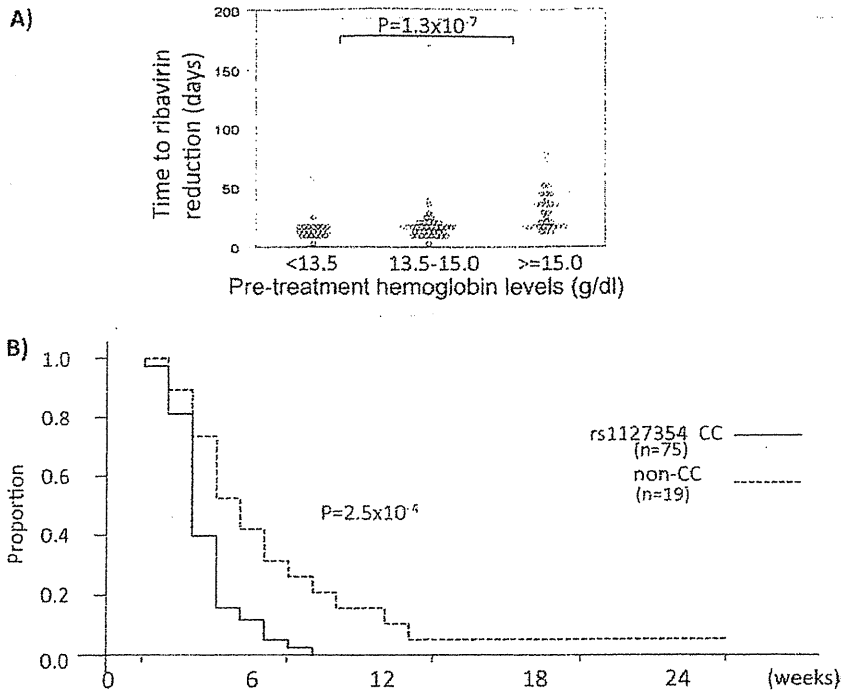

Figure 4. Ribavirin dose reduction during triple therapy. *A*, Number of days of treatment until first ribavirin dose reduction, by pretreatment hemoglobin levels. *B*, Kaplan-Meier curve for dose reduction grouped by ITPA SNP rs1127354 genotype (solid line represents CC; dashed line represents non-CC).

compared with that of naive patients. This is partly because of the higher frequency of the favorable rs8099917 TT genotype among patients who previously experienced relapse (33 [75%] of 44) than among naive patients (15 [60%] of 25), which perhaps reflects the fact that all patients who previously experienced relapse demonstrated at least a transient response to combination therapy and that this group is less likely to include as many patients with non-TT genotypes. All of the treatment-naive patients with the favorable genotype (15 [100%] of 15) achieved SVR, compared with 31 (94%) of 33 patients who previously experienced relapse; conversely, only one-half of the treatment-naive patients with unfavorable rs8099917 genotypes (5 [50%] of 10) achieved SVR, compared with only 1 (9%) of 11 of the patients who previously experienced relapse. This suggests that, although patients who previously experienced relapse have a demonstrated potential to respond to the therapy, there should be more variability among naive patients. Another consideration is that the frequency of the favorable wild-type core70 amino acid was slightly higher among patients who previously experienced relapse (28 [64%] of 44) than among naive patients (13 [52%] of 25). It should be noted, however, that the small number of patients in this study limits the conclusions that can be drawn, and results should be verified in a larger study, perhaps using stratified sampling based on patient background with regard to treatment history to establish more homogeneous patient populations.

In this and a number of other studies, variation in the IL28B locus remains the strongest predictor of SVR reported to date [16–18, 35]. It is unclear which, if any, of the reported SNPs is the primary or functional SNP, but most studies report results for rs8099917 and/or rs12979860, which are under strong linkage disequilibrium in Japanese patients and fall within the intergenic region upstream of IL28B. Although the mechanism is unknown, IL28B and the other 2 members of the IFN-λ family, IL28A and IL29, code for type III IFNs, which are similar to type I IFNs but use a highly tissue-specific receptor [36, 37]. IFN-stimulated genes appear to be initially down-regulated in patients with the favorable rs8099917 TT genotype [38], which may help to prevent desensitization and promote maximal induction of IFN-stimulated genes, although mechanistic studies are needed to understand the connection between IL28B and SVR.

In addition to IL28B polymorphisms, a number of studies have reported that amino acid substitutions in the HCV core protein and the ISDR region of NS5A independently predict treatment outcome after combination therapy [14, 22, 28, 30], and these findings have recently been extended to triple therapy[39, 40]. In this study, substitution at core70 was significant in univariate tests and was selected for inclusion in the multivariate model, but it was not significant in multiple logistic regression. One reason for this may be that core substitutions were initially reported to be associated with nonresponse [22], whereas this study focused on SVR because of the very small number of nonresponders. Terms that are significant in univariate but not multivariate tests may be correlated with each

Table 3. Predictive Factors Associated With SVR in Chronic Hepatitis C Virus Genotype 1 Patients Who Received Pegylated Interferon/Ribavirin/Telaprevir Triple Therapy

	Simple				Multiple			
Variable	*n*	OR	*P*		OR	(95% CI)	*P*	
Treatment-naive	94	1.6	.389					
Previous non-responder	94	0.1	5.5E-08	***	0.17	(.04–.66)	.010	*
Previous relapser	94	10.7	5.2E-05	***				
Age	94	0.8	.939					
Sex (male vs female)	94	1.5	.100					
BMI (kg/m2)	94	0.9	.558					
rs8099917 (TT vs GT/GG)	94	0.1	1.7E-06	***	0.12	(.02–.65)	.014	*
rs1127354 (CC vs AC/AA)	94	1.0	.980					
Core aa70 (wt vs mutant)	93	0.2	.0053	**	0.35	(.09–1.31)	.119	
Core aa91 (wt vs mutant)	93	0.5	.111					
ISDR (0–1 vs ≥2)	90	1.7	.308					
Viral load	94	1.1	.560					
ALT (IU/L)	94	0.9	.142					
gammaGTP	94	0.7	.0009	***				
Hemoglobin (g/dL)	94	1.4	.292					
WBC (/mm^3)	94	1.3	.271					
Platelets (×104/mm^3)	94	1.7	.165					
Total cholesterol (mg/dL)	94	1.7	.160					
LDL cholesterol (mg/dL)	94	2.6	.018	*				
Days to first ribavirin dose reduction	94	1.2	.129					
RVR	94	10.8	4.4E-05	***	14.00	(2.10–93.2)	.006	**
EVR	94	7992.0	.004	**				

NOTE. Results of simple and multiple logistic regression are shown. The multivariate model was constructed using stepwise selection of univariate terms significant at the .05 level. Symbols: * ($P < .05$), ** ($P < .01$), *** ($P < .001$).

other, and only the factor with the strongest effect remains significant. In this case, core70 is significantly correlated with the stronger rs8099917 genotype ($r = .31$; $P = .0027$), although other studies have shown that these terms contribute independently, especially when a larger number of patients are included [39]. Without knowing the mechanism underlying either factor, it is not possible to determine whether the underlying factors that they represent are in fact independent or whether they represent different aspects of a common unknown factor.

Although novel therapies that are not based on IFN and ribavirin are urgently needed, the pending introduction of protease inhibitors represents a pivotal addition to the treatment arsenal, especially for patients who show at least partial response to combination therapy. Because telaprevir is effective as monotherapy, even if only briefly until resistant mutations emerge, alternate combination therapies based on telaprevir and another component designed to raise the barrier to resistance may provide an adequate alternative for older patients and patients unable to tolerate IFN or ribavirin. Furthermore, identification of additional SNPs associated with anemia and other adverse effects will help reduce complications and the need for dose reductions and may lead to treatment guidelines for at-risk patients, such as administration of erythropoietin to stimulate erythropoiesis [41]. Ribavirin dose reductions were required significantly earlier in patients with ITPA SNP genotype CC, compared with patients with non-CC genotypes, which may contribute to poorer response if cumulative ribavirin administration decreases to <80% of the planned dose [26], although ribavirin dose reduction did not affect SVR rate in this study.

In conclusion, triple therapy with PEG-IFN, ribavirin, and telaprevir resulted in higher rates of SVR, compared with PEG-IFN plus ribavirin combination therapy, especially among treatment-naive patients and patients who showed transient response to prior treatment. ITPA polymorphisms predict ribavirin-induced anemia but are not associated with SVR, whereas IL28B polymorphisms and early viral kinetics remain the strongest predictors of SVR with use of triple therapy. Considering both host and viral factors, we identified 2 subgroups of patients who responded well to triple therapy: patients with the favorable rs8099917 TT genotype (47 [94%] of 50) and patients with non-TT genotypes who had wild-type core70 and core91 amino acids (7 [78%] of 9). Patients matching these conditions would benefit most from this 24-week triple therapy, whereas a longer duration of therapy should perhaps be considered for the remaining difficult-to-treat patients.

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References

1. Alter MJ. Epidemiology of hepatitis C in the West. Semin Liver Dis **1995**; 15:5–14.
2. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis **2005**; 5:558–67.
3. Chevaliez S, Pawlotsky JM. Hepatitis C virus: virology, diagnosis and management of antiviral therapy. World J Gastroenterol **2007**; 13:2461–6.
4. Hadziyannis SJ, Sette H Jr, Morgan TR, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med **2004**; 140:346–55.
5. Jang JY, Chung RT. New treatments for chronic hepatitis C. Korean J Hepatol **2010**; 16:263–77.
6. Fowell AJ, Nash KL. Telaprevir: a new hope in the treatment of chronic hepatitis C. Adv Ther **2010**; 27:512–22.
7. Foy E, Li K, Wang C, et al. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. Science **2003**; 300:1145–8.
8. Reesink HW, Zeuzem S, Weegink CJ, et al. Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase Ib, placebo-controlled, randomized study. Gastroenterology **2006**; 131:997–1002.
9. Sarrazin C, Kieffer TL, Bartels D, et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. Gastroenterology **2007**; 132:1767–7.
10. McHutchison JG, Everson GT, Gordon SC, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med **2009**; 360:1827–38.
11. McHutchison JG, Manns MP, Muir AJ, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med **2010**; 362:1292–303.
12. Suzuki F, Suzuki Y, Akuta N, et al. Sustained virological response in a patient with chronic hepatitis C treated by monotherapy with the NS3-4A protease inhibitor telaprevir. J Clin Virol **2010**; 47:76–8.
13. Dienstag JL, McHutchison JG. American Gastroenterological Association technical review on the management of hepatitis C. Gastroenterology **2006**; 130:231–64; quiz 214–7.
14. Akuta N, Suzuki F, Kawamura Y, et al. 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**; 46:403–10.
15. Romero-Gómez M, Del Mar Viloria M, Andrade R, et al. Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. Gastroenterology **2005**; 128:636–41.
16. Ge DL, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature **2009**; 461:399–401.
17. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet **2009**; 41:1105–9.
18. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet **2009**; 41:1100–U74.
19. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature **2009**; 461:798–801.
20. Zeuzem S, Franke A, Lee JH, Herrmann G, Ruster B, Roth WK. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. Hepatology **1996**; 24:1003–9.
21. Enomoto N, Sakuma I, Asahina Y, et al. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis-C virus 1b—sensitivity to interferon is conferred by amino-acid substitutions in the NS5A region. J Clin Invest **1995**; 96:224–30.
22. Akuta N, Suzuki F, Sezaki H, et al. 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 **2005**; 48:372–80.
23. Fellay J, Thompson A, Ge D, et al. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature **2010**; 464:405–8.
24. Thompson A, Fellay J, Patel K, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. Gastroenterology **2010**; 139:1181–9.
25. Ochi H, Maekawa T, Abe H, et al. ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy—a genome-wide study of Japanese HCV virus patients. Gastroenterology **2010**; 139:1190–7. e3.
26. McHutchison J, Manns M, Patel K, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology **2002**; 123:1061–9.
27. Kumada H, Okanoue T, Onji M, et al. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. Hepatol Res **2010**; 40:8–13.
28. Akuta N, Suzuki F, Sezaki H, et al. 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 **2006**; 78:83–90.
29. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis–diagnosis, grading and staging. Hepatology **1994**; 19:1513–20.
30. 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. New Engl J Med **1996**; 334:77–81.
31. Rauch A, Kutalik Z, Descombes P, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology **2010**; 138:1338–45, 1345 e1–7.
32. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet **2001**; 46:471–7.
33. Suzuki A, Yamada R, Chang X, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet **2003**; 34:395–402.
34. Poordad F, Reddy KR, Martin P. Rapid virologic response: a new milestone in the management of chronic hepatitis C. Clin Infect Dis **2008**; 46:78–84.
35. Thompson AJ, Muir AJ, Sulkowski MS, et al. IL28B polymorphism improves viral kinetics and is the strongest pre-treatment predictor of SVR in HCV-1 patients. Gastroenterology **2010**; 139:120–9.e18.
36. Marcello T, Grakoui A, Barba-Spaeth G, et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Gastroenterology **2006**; 131:1887–98.
37. Kotenko SV, Gallagher G, Baurin VV, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol **2003**; 4:69–77.

38. Honda M, Sakai A, Yamashita T, et al. Hepatic ISG expression is associated with genetic variation in IL28B and the outcome of IFN therapy for chronic hepatitis C. Gastroenterology 2010; 139:499–509.
39. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 2010; 52:421–9.
40. Akuta N, Suzuki F, Hirakawa M, et al. Amino acid substitutions in the hepatitis C virus core region of genotype 1b affect very early viral dynamics during treatment with telaprevir, peginterferon, and ribavirin. J Med Virol 2010; 82:575–82.
41. Dieterich D, Wasserman R, Bräu N, et al. Once-weekly epoetin alfa improves anemia and facilitates maintenance of ribavirin dosing in hepatitis C virus-infected patients receiving ribavirin plus interferon alfa. Am J Gastroenterol 2003; 98:2491–9.

INTERNAL MEDICINE

ORIGINAL ARTICLE

Efficacy and Safety of Combination Therapy of Natural Human Interferon Beta and Ribavirin in Chronic Hepatitis C patients

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Abstract

Objective The aim of this study was to evaluate the efficacy and safety of combination therapy of natural human interferon-beta and ribavirin for patients for whom prior interferon therapy was discontinued due to depression induced by interferon-alpha.

Methods Inclusion criteria were as follows; 1) HCV-genotype 1b, 2) serum HCV RNA level of $\geq$100 KIU/mL, 3) stopping the prior interferon-alpha monotherapy or combination therapy of interferon-alpha and ribavirin due to the appearance of depression. A total of 14 were enrolled in this prospective cohort study. The treatment period of combination therapy was 48 weeks. Depression states, reflected by Beck depression inventories and Hamilton depression rating scale, were assessed during combination therapy. Nonparametric procedures were employed for the analysis of background features of the patients with sustained virological response (SVR) and without SVR. A p value of <0.05 was considered to indicate a significant difference.

Results Five of 14 patients (37.5%) had SVR by the intention to treat analysis. The SVR rate in patients who showed negative HCV RNA at 12 and 24 weeks after the initiation of combination therapy was 100% (4/4) and 83.3% (5/6), respectively. All of the patients continued the combination therapy owing to disappearance of severely adverse events contained the exacerbation of depression. Combination therapy did not yield a statistical difference in Beck depression inventories and Hamilton depression rating scale.

Conclusion The combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

Key words: chronic hepatitis C, depression, natural interferon-beta, ribavirin, HCV genotype 1b

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Introduction

The combination therapy of peginterferon-alpha and ribavirin has been widely recommended as a first choice for chronic hepatitis C patients with high virus-load (1-5). However, one big problem of the combination therapy is the treatment-related side effect (6, 7). In particular, physicians in charge tend to avoid the combination therapy of peginterferon-alpha and ribavirin for chronic hepatitis C patients with depression or interferon (IFN)-reduced depression.

IFN-beta-related side effects are mild and few compared to therapy of IFN-alpha (6-8). In particular, IFN-beta-induced mental disorders are mild compared to those induced by IFN-alpha (9). Moreover, IFN-beta could be given to elderly patients aged $\geq$70 years because of the mild side effects (10). However, IFN-beta monotherapy does not result in a satisfactory outcome in patients with genotype 1b and a high virus load (11, 12). The combination therapy of IFN-

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beta and ribavirin has the possibility to show the strong effect for hepatitis C virus (HCV) and mild side effects originating from the treatment (13-15). We have reported that the combination of IFN-beta plus ribavirin therapy is effective and safety for HCV patients with high virus load and depressive state (14). However, the previous study was retrospective and a prospective study is necessary to evaluate the efficacy and safety of combination therapy of IFN-beta and ribavirin for HCV patients with high virus load and depressive state.

Thus, in the present study, we performed a prospective study to examine the efficacy and safety of combination therapy of IFN-beta and ribavirin in HCV genotype 1b patients who had stopped the IFN therapy due to depression induced by IFN-alpha. At the same time, depression states, reflected by Beck depression inventories (BDI) and Hamilton depression rating scale (Ham-D), were assessed during combination therapy (16, 17).

Materials and Methods

Patients

Eligibility criteria for entry into the study included the following: 1) HCV genotype 1b; 2) serum level of HCV RNA of ≥100 KIU/mL before treatment; 3) stopping of IFN-alpha therapy due to depression appearance during the prior IFN-alpha treatment; 4) Ham-D of <18; 5) no corticosteroid, immunosuppressive agents, or antiviral agents used within 6 months; 6) no hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) detectable in serum, determined by radioimmunoassay; 7) white blood cell (WBC) >2,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/mL; follow up for >6 months before treatment. We excluded from the study all of the patients with the following: 1) a history of alcohol abuse; 2) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. The physician in charge explained the purpose and method of the combination therapy of IFN-beta and ribavirin as well as the potential adverse reactions to each patient and informed consent was obtained from each patient. This study was approved by the Human Ethics Review Committee of Toranomon Hospital.

From December 2007 to May 2008, 14 HCV patients were enrolled in this prospective cohort study at the study hospital. A sustained virological response (SVR) was defined as clearance of HCV RNA by commercial amplicor HCV qualitative assay (Amplicor HCV; Ver.2.0, Roche Diagnostic Systems, Basel, Switzerland) at 6 months after the cessation of combination therapy (18).

Laboratory investigation

Blood samples were obtained just before and 6 month after combination therapy. The samples were stored at -80℃ until analysis. Using these blood samples, HCV-RNA level before IFN therapy was analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) (19). Negativity of serum HCV RNA was defined as clearance of serum HCV RNA by commercial amplicor HCV qualitative assay (18). 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 (20). The core protein of HCV-1b was determined by the previous report (21). Next, the genetic variations near the IL28B gene (rs8099917), reported as the pretreatment predictors of treatment efficacy and clinical outcome, were investigated (22-26). Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) concentrations, and HCV RNA were measured at least once per month during therapy. Clinical evaluation and biochemical and hematological tests were performed at 1, 2, and 4 weeks in the first month after the initiation of combination therapy. After that, these evaluations were done at monthly intervals. The patients were followed by both physicians of hepatology and psychiatry.

Combination therapy of IFN-beta and ribavirin

Treatment was provided for 48 weeks. IFN-beta (Feron, Toray Industries Inc., Tokyo, Japan) was given intravenously at a dose of 6 million units (MU) by six times a week for 4 weeks, followed by three times a week for 44 weeks. The total dose was 936MU. Ribavirin (Rebetol, MSD KK., Tokyo, Japan) was given at the dose prescribed based on body weight. The ribavirin dose was adjusted according to body weight (600 mg for ≤60 kg, 800 mg for >60 kg and ≤80 kg, and 1,000 mg for >80 kg).

Evaluation of the psychic state

The psychiatrist in charge evaluated the scores of BDI and Ham-D prospectively. BDI shows the subjective symptom of the depressive patients and Ham-D shows the objective evaluation by the psychiatrist. Scores on the BDI were divided the following; severe, 29-63; moderate, 20-28; mild, 14-19; and minimal, 0-13. Scores on the Ham-D were divided the following; very severe, >23; severe, 19-22; moderate, 14-18; mild, 8-13; and normal ≤7 (27).

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 and Fisher's exact test. The following variables were evaluated as prognostic factors: sex, age, BDI score, Ham-D score, a HCV RNA level, IL28B (genetic variation in rs8099917), variation of HCV-core, biochemical factors (AST, ALT, gamma glutamyltransferase, total cholesterol), white blood cell (WBC), hemoglobin, platelet count, HCV RNA 4, 12, 24 week after the initiation of IFN therapy. The SPSS software package (SPSS Inc., Chicago, IL) was used to perform statistical analysis. A p value of <0.05 was considered to indicate a significant difference.

Table 1. **The Difference of Clinical Backgrounds between Patients with SVR and Those without SVR ***

	Total	SVR (n=5)	Non-SVR (n=9)	p value†
Age (years old)	62.1 ± 4.3	62.4 ± 4.2	61.9 ± 4.6	0.797
Sex (male/female)	6/8	2/3	4/5	0.898
Previous IFN therapy (combination/monotherapy)	8/6	3/2	5/4	0.898
Duration of previous IFN therapy (week)	11.9 ± 7.8	11.6 ± 10.2	12.0 ± 7.1	0.699
HCV-RNA (KIU/mL)	2588± 1455	2228± 1807	2788± 1296	0.759
Core aa70 (Wild/Mutant)	6/8	3/2	3/6	0.438
BDI score	11.9 ± 10.3	12.2 ± 14.2	11.7 ± 8.4	0.518
Ham-D score	3.5 ± 4.1	3.6 ± 5.5	3.4 ± 3.5	0.606
IL28B (genetic variation in rs8099917, genotype TT/TGorGG)	7/7	5/0	2/7	0.042
AST (IU/L)	50 ± 24	46 ± 37	52 ± 17	0.112
ALT (IU/L)	68 ± 33	60 ± 35	72 ± 32	0.518
GGT (IU/L)	55 ± 59	25 ± 5	72 ± 69	0.813
Total cholesterol (mg/dL)	175 ± 30	166 ± 35	179 ± 28	0.298
White blood cell(10^3/mm^3)	4.39 ± 1.24	4.16 ± 1.02	4.52 ± 1.39	0.898
Hemoglobin (g/dL)	14.1 ± 1.1	14.2 ± 1.5	14.0 ± 0.9	0.898
Platelet(10^4/mm^3)	15.8 ± 4.8	19.9 ± 2.4	13.5 ± 4.1	0.019
HCV RNA (+/-) 4W	11/3	2/3	9/0	0.083
HCV RNA (+/-) 12W	10/4	1/4	9/0	0.012
HCV RNA (+/-) 24W	8/6	0/5	8/1	0.004

Data are number of patients (percentage) or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDI, Beck depression inventories; GGT, gamma-glutamyltransferase; Ham-D, Hamilton depression rating; HCV, hepatitis C virus;
*IFN-beta was given intravenously at a dose of 6 million units (MU) daily for 4 weeks, followed by three times a week for 44 weeks.
†Nonparametric procedures were employed for the analysis of background features of the patients with SVR and without SVR, including the Mann-Whitney U test and Fisher' exact test.

Result

Clinical characteristics of the patients

A total of 14 patients treated with IFN-beta +ribavirin were enrolled in the present study. Table 1 shows the characteristics of the patients who received combination therapy. Clinical profiles were as follows: mean age =62.1 years, male/female =6/8, and HCV-RNA =2,588±1,455 KIU/mL. Patients were classified into two groups according to the difference of response: SVR (n=5), Non-SVR (n=9).

Efficacy of treatment

Five of 14 patients (37.5%) had SVR by the intention to treat analysis. Table 1 shows the differences in the clinical background between patients with SVR and those without SVR. The negativity rate of HCV RNA 12 weeks after the initiation of combination therapy was 80% (4/5) in SVR group and 0%(0/9) in Non-SVR group (p=0.012). The negativity rate of HCV RNA 24 weeks after the initiation of combination therapy was 100% (5/5) in SVR group and 11.1% (1/9) in Non-SVR group (p=0.004). Next, the platelet count in SVR group was significantly higher than that in Non-SVR group.

On the IL28B (genetic variation in rs8099917), all seven patients with TG or GG at IL28B showed non-SVR. On the other hand, five of the seven patients with TT at IL28B showed SVR. The TT at IL28B that is associated with SVR was statistically significant in the present study (p=0.042).

Safety and tolerance of combination therapy

Of the 14 patients treated with IFN-beta + ribavirin included in this study, four patients necessitated a reduced dose of ribavirin due to the appearance of hemoglobin level <10 g/dL and two patients needed a reduced dose of IFN-beta due to WBC count of <2,000/mm^3. Three patients had dipstick proteinuria of +1 at 4 week after the initiation of combination therapy. This proteinuria continued during combination therapy. However, no patient discontinued combination therapy because of treatment related adverse events related to exacerbation of depression. Fig. 1 shows the changes of BDI scores in 14 patients treated with IFN-beta + ribavirin. BDI scores during combination therapy were lower than that at the initiation time of treatment. Fig. 2 shows the changes of Ham-D scores in 14 patients. There was no statistically significant difference in changes of Ham-D scores during combination therapy compared to that at the initiation time of treatment.

Regarding the prescription of antidepressant and anti-anxiety drugs, antidepressants, such as sulpiride, and amitriptyline hydrochloride, were given to three patients at the

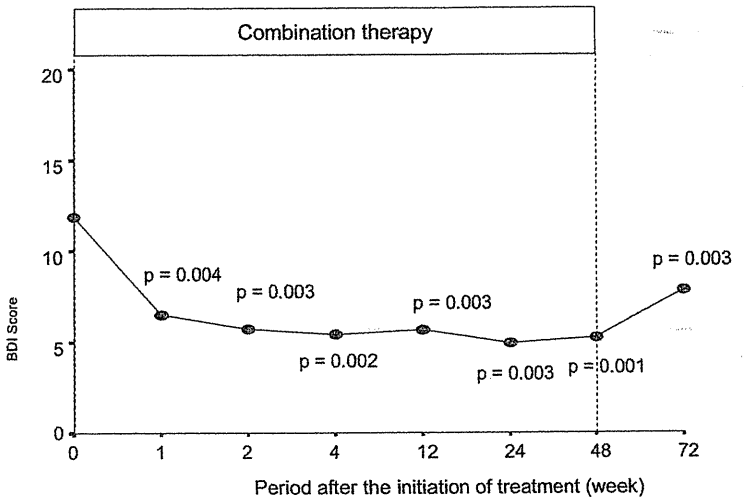

Figure 1. **The change of BDI score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the BDI-2 score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

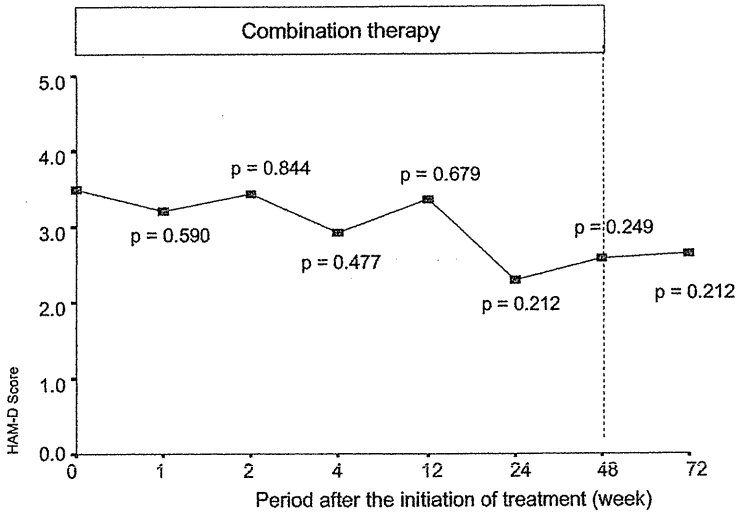

Figure 2. **The change of Ham-D score after the initiation of combination therapy. P-values at 1, 2, 4, 12, 24, 48, and 72 weeks indicate the statistical difference compared with the HAM-D score at the initiation time of combination therapy by the use of Mann-Whitney U test.**

start of IFN therapy and to four patients during IFN therapy. Anti-anxiety drugs, such as etizolam, alprazolam, were given to four patients at the start of IFN therapy and to five patients during IFN therapy.

The changes of WBC, hemoglobin, and platelet count after the initiation of combination therapy are shown in Fig. 3. WBC and hemoglobin levels were decreased during combination therapy. On the other hand, the platelet count decrease was statistically significant at 1, 2, and 4 weeks after the initiation of combination therapy compared to that at the initiation time of treatment. After that, the platelet count recovered to the base line at 12, 24, and 48 weeks after the initiation of combination therapy.

Discussion

In the present study, we have described the efficacy and safety of combination therapy of IFN-beta and ribavirin for patients for whom IFN therapy was discontinued due to depression induced by IFN-alpha. The patients with HCV genotype 1b and HCV-load of $\geq$100 KIU/mL were enrolled. We could evaluate the relationship between IL-28 or HCV core mutation and SVR in the combination therapy of IFN-beta and ribavirin for genotype 1b and high virus load. The present study was limited to exclude the subjects with Ham-D score of more than 18. Patients with Ham-D score of more than 18 were defined as severe depression state. It is possible that high score of Ham-D enhance the dropout

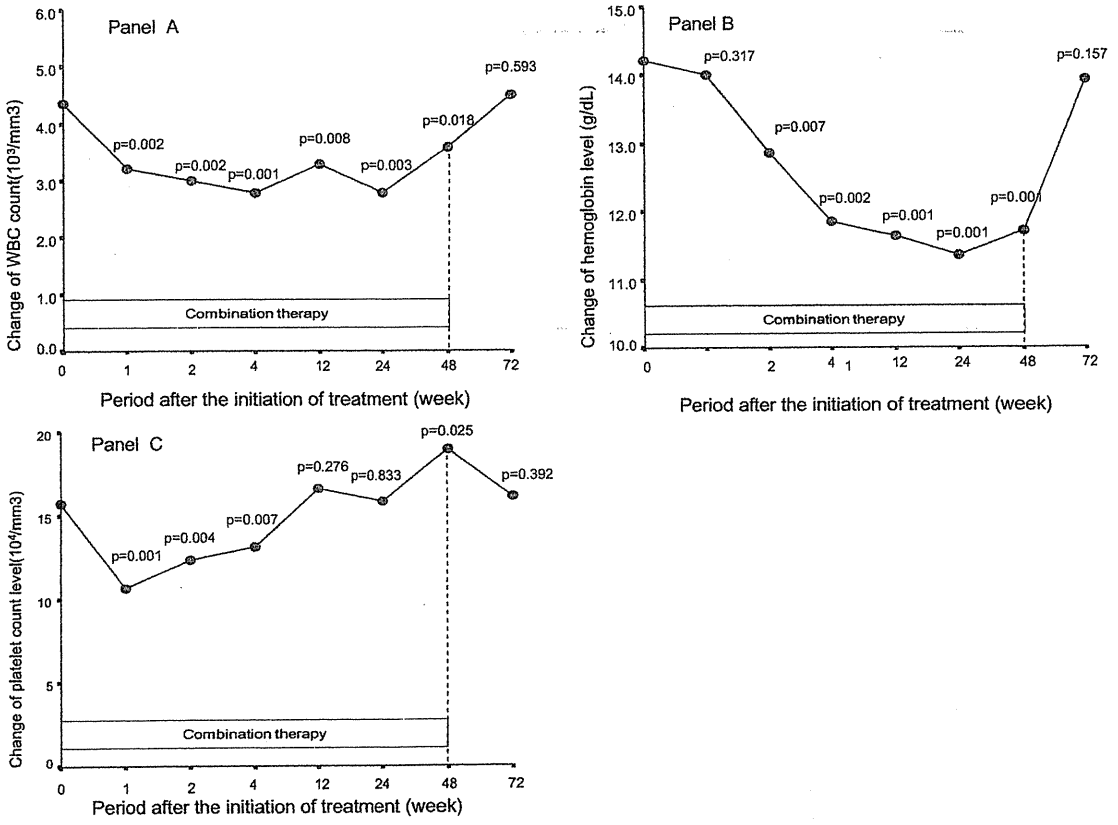

Figure 3. **The change of complete blood cell count after the initiation of combination therapy. Panel A; The change of white blood cell count. Panel B; The change of hemoglobin level. Panel C; The change of platelet count.**

due to combination therapy and aggravation of depressive state. Thus, we excluded the patients with Ham-D score of more than 18 in the present study. Moreover, the number of 14 patients enrolled was a small size. Another limitation is that the present study was not a randomized controlled study. Several findings from the present study have direct implications for combination therapy of IFN-beta and ribavirin for chronic hepatitis C in the future. First, the drop-out rate due to depressive state in combination therapy of IFN-beta and ribavirin was low. This result was similar to that in the previous study (14). The result by this prospective study confirmed that combination therapy of IFN-beta and ribavirin reduced the aggravation of depressive state compared with combination therapy of peginterferon-alpha and ribavirin.

Second, 5 out of 14 patients treated with combination therapy of IFN-beta and ribavirin had SVR. The SVR rate in the present study was almost the same to that in the previous study.

Third, SVR had a tendency to occur in patients with negativity of HCV RNA at 12 and/or 24 weeks after the initiation of combination therapy. All of the patients with positive HCV RNA at 24 weeks after the initiation of combination therapy showed non-SVR. This result agreed with our previous report (14). Thus, positive HCV RNA at 24 weeks after the initiation of combination therapy of IFN-beta and ribavirin suggests that the possibility of SVR is low. Next, patients with a high platelet count tended to show SVR. In general, a high platelet count suggests slight fibrosis of liver. Thus, the result raises the possibility that slight hepatic fibrosis enhance the efficacy of combination therapy.

Finally, SVR in combination therapy of IFN-beta + ribavirin was associated with IL-28B in the present study. None of the seven patients with genotype TG or GG at the genetic variation in rs8099917 near the IL28B gene had SVR. The results suggested that only patients with genotype TT might have the possibility of getting SVR. On substitution of core amino acid (aa) 70, two of eight patients with mutant type of core aa 70 showed SVR. The result shows that patients with mutant type of core aa 70 have the possibility of getting SVR. Several authors have reported that virus clearance in combination therapy of peginterferon-alpha and ribavirin is associated with HCV mutations in the core region and IL-28B (21-26). The present study confirmed that IL-28B was related with SVR for HCV patients with genotype 1b and high virus load.

IFN-beta is not convenient for treatment compared to intramuscular or subcutaneous injection. However, IFN-beta-related side effects are mild and few compared to those of IFN-alpha. IFN-beta-induced mental disorders are mild compare to those induced by IFN-alpha. Out of 7,250 HCV patients treated with IFN in our hospital, 960 (13.2%) were

given IFN-beta. The mechanism of the better tolerability of IFN-beta and ribavirin is unclear. However, the following mechanism might be considered: 1) IFN-beta is not recombinant IFN but produced from human white blood cell. Thus, IFN-beta has a tendency not to produce some immune complex relating to IFN-related side effects. 2) IFN-beta might have different intracellular mechanisms compared to IFN-alpha. Although the receptor of IFN alpha and beta are common, intracellular mechanisms could differ. Our results described above suggest that combination therapy of IFN-beta and ribavirin is one possible method for patients who have HCV-genotype 1, high virus load and depressive state of Ham-D scale of <18. In conclusion, the combination therapy of IFN-beta and ribavirin is a possible therapy selection for the patients for whom interferon therapy was discontinued due to depression induced by interferon-alpha.

The authors state that they have no Conflict of Interest (COI).

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References

1. Manns MP, McHutchison JG, Gordon SC, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet **358**: 958-965, 2001.
2. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med **347**: 975-982, 2002.
3. McHutchison JG, Manns M, Patel K, et al; International Hepatitis Interventional Therapy Group. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology **123**: 1061-1069, 2002.
4. Hadziyannis SJ, Sette H, Morgan TR, et al; 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 **140**: 346-355, 2004.
5. Shiffman ML, Ghany MG, Morgan TR, et al. Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. Gastroenterology **132**: 103-112, 2007.
6. Iwasaki Y, Ikeda H, Araki Y, et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology **43**: 54-63, 2006.
7. Arase Y, Suzuki F, Suzuki Y, et al. Side effects of combination therapy of peginterferon and ribavirin for chronic hepatitis-C. Intern Med **46**: 1827-1832, 2007.
8. Festi D, Sandri L, Mazzella G, et al. Safety of interferon beta treatment for chronic HCV hepatitis. World J Gastroenterol **10**: 12-16, 2004.
9. Katamura Y, Suzuki F, Akuta N, et al. Natural human interferon beta plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus and a high viral load. Intern Med **47**: 1827-1834, 2008.
10. Arase Y, Suzuki F, Suzuki Y, et al. The efficacy of interferon-beta monotherapy for elderly patients with type C hepatitis of genotype 2. Intern Med **48**: 1337-1342, 2009.
11. Kainuma M, Ogata N, Kogure T, et al. The efficacy of a herbal medicine (Mao-to) in combination with intravenous natural interferon-beta for patients with chronic hepatitis C, genotype 1b and high viral load: a pilot study. Phytomedicine **9**: 365-372, 2002.
12. Kurosaki M, Enomoto N, Murakami T, et al. Analysis of genotypes and amino acid residues 2209 to 2248 of the NS5A region of hepatitis C virus in relation to the response to interferon-beta therapy. Hepatology **25**: 750-753, 1997.
13. Enomoto M, Tamori A, Kawada N, et al. Interferon-beta plus ribavirin for patients with hepatitis C virus genotype 1: a randomized pilot trial. Gut **55**: 139-140, 2006.
14. Arase Y, Suzuki F, Akuta N, et al. Efficacy and safety of combination therapy of natural human interferon beta and ribavirin in chronic hepatitis C patients with genotype 1b and high virus load. Intern Med **49**: 957-963, 2010.
15. Arase Y, Suzuki F, Akuta N, et al. Efficacy and safety of combination therapy of natural human interferon Beta and ribavirin in chronic hepatitis C patients with genotype 2 and high virus load. Intern Med **49**: 965-970, 2010.
16. Beck AT. Comparison of Beck Depression Inventories-IA and -II in psychiatric outpatients. J Pers Assess **67**: 588-597, 1996.
17. Hamilton M. A rating scale for depression. J Nerol Neurosurg Psychiat **23**: 56-62, 1960.
18. Doglio A, Laffont C, Caroli-Bosc FX, Rochet P, Lefebre 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. 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.
20. 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.
21. Akuta N, Suzuki F, Sezaki H, et al. Association of amino acid substitution pattern in core protein of hepatitis C virusgenotype1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology **48**: 372-380, 2005.
22. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature **461**: 399-401, 2009.
23. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet **41**: 1105-1109, 2009.
24. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet **41**: 1100-1104, 2009.
25. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28 B and spontaneous clearance of hepatitis C virus. Nature **461**: 798-801, 2009.
26. Rauch A, Kutalik Z, Descombes P, et al; Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. Swiss Hepatitis C and HIV Cohort Studies. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure—a genome-wide association study. Gastroenterology **138**: 1338-1345, 2010.
27. Kearns NP, Cruickshank CA, McGuigan KJ. A comparison of depression rating scales. Br J Psychiatry **141**: 5-49, 1982.

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Amino Acid Substitutions in Hepatitis C Virus Core Region Predict Hepatocarcinogenesis Following Eradication of HCV RNA by Antiviral Therapy

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Substitution of amino acid (aa) 70 and/or 91 in the core region of HCV genotype 1b (HCV-1b) is an important predictor of hepatocarcinogenesis, but its impact on the development of hepatocellular carcinoma (HCC) following eradication of HCV RNA by antiviral therapy is not clear. 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of interferon monotherapy or interferon plus ribavirin combination therapy, were included in a follow-up study to evaluate the impact of aa substitution in the core region on hepatocarcinogenesis. Twenty six patients developed HCC during the follow-up. The cumulative rates of new HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively. The rates in patients infected with HCV-1b/Gln70(His70) [glutamine (histidine) at aa 70] were significantly higher than in patients infected with HCV-1b/Arg70 (arginine at aa 70) ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). The rates in patients infected with HCV-1b/Arg70 were not significantly higher than in those infected with HCV-2a/2b ($P = 0.617$; log-rank test). Multivariate analysis identified HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced fibrosis (HR 9.03, $P = 0.002$), and old age (HR 3.09, $P = 0.066$) as determinants of hepatocarcinogenesis. In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of HCC following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy. ***J. Med. Virol. 83:1016–1022, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: HCV; genotype; sustained virological response; hepatocellular carcinoma; core region; glutamine

INTRODUCTION

Infection with hepatitis C virus (HCV) is often persistent and can progress to chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. At present, interferon (IFN), in combination with ribavirin, is the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) and high viral loads account for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Tsubota et al., 2005].

Despite numerous lines of epidemiological evidence of an association between HCV infection and the development of HCC, it remains controversial whether the virus itself plays a direct role or an indirect role in the pathogenesis of HCC [Koike, 2005]. It has become evident that the HCV core region is potentially oncogenic in transgenic mice, but the clinical impact of the core region on hepatocarcinogenesis is still unclear [Moriya et al., 1998]. Previous reports indicated that amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b are pretreatment predictors of poor virological response to pegylated IFN (PEG-IFN)/ribavirin combination therapy and triple therapy of

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telaprevir/PEG-IFN/ribavirin [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also affect hepatocarcinogenesis [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. These reports support the oncogenic potential of the core region from the clinical aspect. However, hepatocarcinogenesis still occurs even after eradication of HCV RNA by antiviral therapy [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008], though whether substitutions of aa 70 and/or 91 in the core region also affect hepatocarcinogenesis following eradication of HCV RNA await further investigation.

The present study included 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy). The aims of this study were to evaluate the impact of aa substitutions in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

PATIENTS AND METHODS

Patients

Among 4,570 consecutive patients infected with HCV, in whom antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy) was initiated between February 1987 and June 2010 at the Toranomon Hospital, 1,273 were selected for the present study. We included patients who fulfilled the following criteria: (1) Patients positive for anti-HCV (by a third-generation enzyme immunoassay, Chiron Corp., Emerville, CA) and for HCV RNA by qualitative or quantitative analysis, before antiviral therapy. (2) Patients with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy, based on HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim, Germany) or by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (3) Patients without HCC, before and during IFN therapy. (4) Patients infected with a single genotype of HCV-1b, 2a, or 2b. (5) Patients negative for hepatitis B surface antigen (by radioimmunoassay, Dainabot, Tokyo). (6) Patients free of coinfection with the human immunodeficiency virus. (7) Lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake). (8) Patients free of other types of hepatitis, and without hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) Each signed a consent form of the study protocol that had been approved by the human ethics review committee.

Table I summarizes the profile and laboratory data at the start of antiviral therapy of 1,273 patients with sustained virological response. They included 783 males and 490 females, aged 15–83 years (median, 53 years). The median follow-up time, from the end of antiviral therapy until the last visit, was 1.1 years (range, 0.0–18.0 years).

Laboratory Investigations

Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [Chayama et al., 1993]. HCV RNA was quantitated by branched DNA assay version 2.0 (Chiron Corp.), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems, Inc., Pleasanton, CA), or COBAS TaqMan HCV test (Roche Diagnostics). A high viral load was defined as branched DNA assay value of

TABLE I. Clinical Profile and Laboratory Data at the Start of Antiviral Therapy

Demographic data	
Number of patients	1,273
Sex (male/female)	783/490
Age (years)*	53 (15–83)
Body mass index (kg/m^2)*	22.7 (14.4–38.0)
Laboratory data	
Serum aspartate aminotransferase (IU/L)*	48 (11–1,386)
Serum alanine aminotransferase (IU/L)*	68 (10–2,009)
Total cholesterol (mg/dl)*	168 (79–328)
Fasting plasma glucose (mg/dl)*	93 (69–290)
HCV genotype (1b/2a/2b)*	664/433/176
Level of viremia (high viral load/low viral load)	838/415
Treatment regimen	
IFN monotherapy/IFN plus ribavirin	545/728
Histological findings	
Stage of fibrosis (F1/F2/F3/F4)	508/224/62/47
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 [arginine/glutamine (histidine)]	348/127
Core aa 91 (leucine/methionine)	321/156

The enrolled patients had sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy.
Data are numbers and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

≥1.0 Meq/ml, AMPLICOR GT HCV Monitor ≥100 × 10^3 IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay value of <1.0 Meq/ml, AMPLICOR GT HCV Monitor <100 × 10^3 IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim) was 100 copies/ml, and that of COBAS TaqMan HCV test was 1.2 log IU/ml. Samples with undetectable HCV RNA at 24 weeks after cessation of antiviral therapy by qualitative analysis or COBAS TaqMan HCV test were defined as HCV RNA-negative.

Detection of Amino Acid Substitutions in the Core Regions of HCV-1b

In the present study, aa substitutions in the core region of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of antiviral therapy and reverse transcribed with a random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids of the core region were amplified by nested PCR using the following primers. The first-round PCR was performed with CE1 (sense, 5′-GTC TGC GGA ACC GGT GAG TA-3′, nucleotides: 134–153) and CE2 (antisense, 5′-GAC GTG GCG TCG TAT TGT CG-3′, nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense, 5′-ACT GCT AGC CGA GTA GTG TT-3′, nucleotides: 234–253) and CE6 (antisense, 5′-GGA GCA GTC GTT CGT GAC AT-3′, nucleotides: 934–953) primers. All samples were initially denatured at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

Using HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed using 50 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. Thus, patients were classified into three HCV subgroups according to the HCV genotype and aa substitutions in the HCV-1b core region: (1) HCV-1b with Arg70, (2) HCV-1b with Gln70(His70), and (3) HCV-2a/2b.

Liver Histopathological Examination

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan). The samples were fixed in 10% formalin and then stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. Each specimen submitted for examination contained ≥6 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 the scoring system of Desmet et al. [1994] for histopathological assessment.

Follow-Up and Diagnosis of Hepatocellular Carcinoma

Hematological, biochemical, and virological tests were performed at least once every month until the virological response was determined. When sustained virological response was confirmed, blood tests and imaging studies (computed tomography or ultrasonography) were conducted once or twice per year in the majority of patients, except those lost to follow-up. When HCC was suspected, additional procedures, such as magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy when necessary, were used to confirm the diagnosis.

Statistical Analysis

The cumulative rate of new cases of HCC was calculated using the Kaplan–Meier technique, and differences between the curves were tested using the log-rank test. Differences in the proportion of new cases of HCC according to groups were analyzed according to the period between the end of antiviral therapy and appearance of HCC. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with the development of HCC. The hazard ratio (HR) and 95% confidence interval (95%CI) were also calculated. Potential predictive factors associated with the development of HCC included the following variables: sex, age, body mass index, AST, ALT, total cholesterol, fasting plasma glucose, HCV genotype, level of viremia, treatment regimen, stage of fibrosis, and HCV subgroup according to HCV genotype in combination with aa substitutions in the core region. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL). All P values of less than 0.05 by the two-tailed test were considered significant.

RESULTS

Rate of New Cases of HCC in Patients With Sustained Virological Response

During the follow-up, 26 patients (2.0%) developed HCC. The median interval between the end of antiviral therapy and detection of HCC (latency to HCC) was 2.5 years (range, 0.0–15.9 years). The cumulative rates of new cases of HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively.

HCC Rate According to HCV Genotype and Amino Acid Substitutions in the Core Region of HCV-1b

During the follow-up, 7 (5.5%), 5 (1.4%), and 12 (2.0%) patients developed HCC in the HCV-1b with Gln70(His70), HCV-1b with Arg70, and HCV-2a/2b groups, respectively. The median latency to HCC was 1.1 years (range, 0.0–14.0 years), 3.9 (range, 0.0–15.9), and 2.8 (range, 0.0–12.9), respectively, and the cumulative rates of new cases of HCC were 10.6%, 3.6%, 3.0% at the end of 5 years; 10.6%, 6.3%, 5.2% at the end of 10 years; and 62.7%, 6.3%, 7.2% at the end of 15 years, respectively. The rates were significantly different among the three HCV subgroups ($P < 0.001$; log-rank test; Fig. 1). Especially, the rates for HCV-1b with Gln70(His70) were significantly higher than those for HCV-1b with Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). However, the rates for the HCV-1b with Arg70 group were not significantly higher than those for the HCV-2a/2b group ($P = 0.617$; log-rank test).

During the follow-up, 4 (2.6%) and 7 (2.2%) patients with HCV-1b/Met91, and HCV-1b/Leu91 developed HCC, respectively. In these two subgroups, the respective median latency to HCC was 3.4 years (range, 0.0–14.0 years) and 1.1 (range, 0.0–12.4), and the cumulative rates of new cases of HCC were 1.3%, 8.6% at the end of 5 years; 5.4%, 8.6% at the end of 10 years; and 36.9%, 14.7% at the end of 15 years. The rates for the HCV-1b/Met91 group were not significantly different from those for the HCV-1b/Leu91 group ($P = 0.908$; log-rank test).

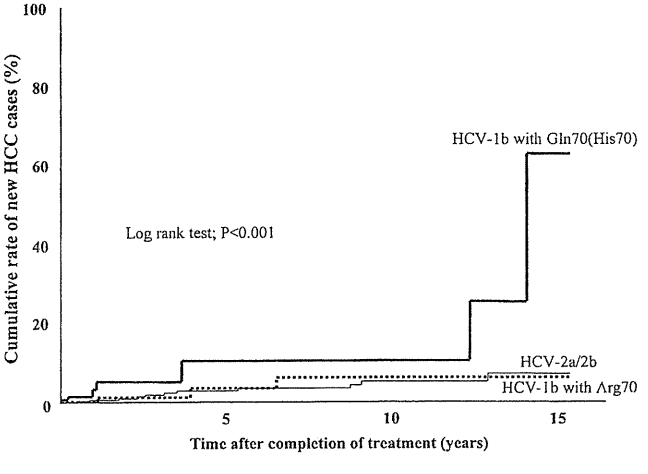

Fig. 1. Cumulative rates of new cases of HCC according to HCV genotype and amino acid substitutions in the core region of HCV-1b. The rates were significantly different among the three HCV groups ($P < 0.001$; log-rank test). Especially, the rate in patients with HCV-1b/Gln70(His70) was significantly higher than those of patients with HCV-1b/Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). Furthermore, the rate in patients with HCV-1b/Arg70 was not significantly higher than that in HCV-2a/2b ($P = 0.617$; log-rank test).

Predictive Factors Associated With the Development of HCC in Patients of Sustained Virological Response

Next, we analyzed the predictor of HCC using data of the entire group. There were significant relationships between the rate of new cases of HCC and male sex ($P = 0.003$), severe fibrosis (F3,4) ($P < 0.001$), old age (≥55 years) ($P = 0.002$), high levels of AST (≥39 IU/L) ($P = 0.023$), and HCV-1b/Gln70(His70) (log-rank test). These five factors were entered into multivariate analysis, which then identified three parameters that independently tended to or significantly influenced the development of HCC; HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced stage of fibrosis (F3,4; HR 9.03, $P = 0.002$), and old age (≥55 years; HR 3.09, $P = 0.066$; Table II).

Predictors of HCC in HCV-1b Patients With Sustained Virological Response

Finally we analyzed the data of 664 patients with HCV-1b to determine the predictors of HCC with sustained virological response. Univariate analysis identified three parameters that significantly correlated with the development of HCC: male sex ($P = 0.005$), old age ($P = 0.020$), and HCV-1b with Gln70(His70) ($P = 0.007$; log-rank test). These three factors were entered into multivariate analysis, which then identified HCV-1b with Gln70(His70) as the single parameter that significantly influenced the development of HCC (HR 8.19, $P = 0.034$).

DISCUSSION

Previous studies reported that the risk factors for hepatocarcinogenesis after elimination of HCV RNA

TABLE II. Results of Multivariate Analysis (Cox Proportional Hazard Model) for Factors Associated With Hepatocarcinogenesis in Patients With Sustained Virological Response

Factors and categories	Hazard ratio (95%CI)	*P*-Value
HCV group		
HCV-2a/2b	1	
HCV-1b with Arg70	1.15 (0.24–5.56)	0.863
HCV-1b with Gln70(His70)	10.5 (2.89–38.2)	<0.001
Fibrosis stage		
F1,2	1	
F3,4	9.03 (2.32–35.2)	0.002
Age (years)		
<55	1	
≥55	3.09 (0.93–10.3)	0.066

were severe fibrosis, male sex, and old age at the start of IFN treatment [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008]. In the present study, multivariate analysis identified HCV-1b with Gln70(His70), advanced fibrosis stage, and old age as determinants of HCC in patients with a sustained virological response. The present study is the first report to indicate that aa substitution in the core region at the start of antiviral therapy also influences hepatocarcinogenesis following eradication of HCV RNA. This result should be interpreted with caution since races other than the Japanese and patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies of patients of other races and those infected with HCV-1a.

Despite numerous lines of epidemiological evidence linking HCV infection to the development of HCC, it remains controversial whether HCV itself plays a direct or indirect role in the pathogenesis of HCC [Koike, 2005]. Evidence suggests that the HCV core region is potentially oncogenic in the transgenic mice [Moriya et al., 1998], though the clinical impact of the core region on hepatocarcinogenesis remains unclear. Previous reports indicated that aa substitutions in the core region of HCV-1b are pretreatment predictors of poor virological response to antiviral therapy [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also are etiological factors in HCC [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. Importantly, the present study indicated that aa substitution in the core region at the start of antiviral therapy also affects the development of HCC even after the eradication of HCV RNA, and this is the first report to suggest the persistent oncogenic potential of the core region regardless of HCV RNA persistence. Previous reports identified the PA28γ-dependent pathway as one of the mechanisms of HCV-associated hepatocarcinogenesis. Moriishi et al. [2003, 2007] reported that knockout of the PA28γ gene induces accumulation of HCV core protein in the nuclei of hepatocytes of HCV core gene transgenic mice and disrupts the development of both hepatic steatosis and HCC. Furthermore, the HCV core protein also enhances the binding of liver X receptor α (LXRα) and retinoid X receptor α (RXRα) to the LXR-response element in the presence of PA28γ [Moriishi et al., 2007]. Thus, it seems that PA28γ plays a crucial role in the development of HCV-associated steatosis and HCC. However, these basic studies were performed under the state of HCV RNA persistence [Moriya et al., 1998; Moriishi et al., 2003, 2007; Koike, 2005], and further studies should be performed to investigate the oncogenic potential of aa substitution in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

The association between HCV genotype and the risk of HCC is not clear. A study of Italian cohort indicated that the rate of HCC in patients infected with HCV-1b was significantly higher than that of patients infected with HCV-2a/2c [Bruno et al., 2007]. On the other hand, the present study of Japanese patients indicated that the rates in patients infected with HCV-1b were not significantly higher than those in those infected with HCV-2a/2b. The discrepancy between the present result and the above Italian study may be explained by differences in host factors [Montes-Cano et al., 2010], and/or differences in viral factors, such as the distribution of HCV-1b with Arg70 or Gln70(His70), and geographic diversities of HCV-1b [Nakano et al., 1999].

Previous studies showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin achieved sustained virological response rates of 35–60% and 61–69% in patients infected with HCV-1, respectively [Hézode et al., 2009; McHutchison et al., 2009; Akuta et al., 2010]. Furthermore, the PROVE3 study also showed that the 24- and 48-week regimen of triple therapy achieved sustained virological response rates of 51% and 53%, respectively, in patients infected with HCV-1 who had been unsuccessfully treated with PEG-IFN/ribavirin [McHutchison et al., 2010]. While it is anticipated that larger numbers of HCV-1 patients will achieve sustained virological response in response to telaprevir/PEG-IFN/ribavirin, a larger proportion of patients could develop HCC following eradication of HCV RNA by antiviral therapy. Hence, our study indicated that aa substitutions in the core region of HCV-1b should be detected before eradication of HCV RNA by antiviral therapy. Especially, even if patients of HCV-1b with Gln70(His70) could achieve sustained virological response, blood tests and imaging studies should be conducted at regular intervals in this high risk group for early detection and treatment of HCC.

Genetic variations near the IL28B gene are pretreatment predictors of poor virological response to the combination therapy of PEG-IFN/ribavirin and triple therapy of telaprevir/PEG-IFN/ribavirin [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Akuta et al., 2010; Rauch et al., 2010], but their impact on hepatocarcinogenesis are unknown at this stage. In this study, 387 of 1,273 patients were evaluated for HCC according to genetic variation in rs8099917 (data not shown). A preliminary study based on a small number of patients showed that the HCC rate in genotype TT of treatment sensitive type (2.2%) was not significantly different from that in genotype non-TT of treatment resistant type (1.6%). Unfortunately, we could analyze the effect of rs8099917 on HCC following eradication of HCV RNA by antiviral therapy. Further studies of larger patient populations should be performed to investigate the relationship between genetic variations near the IL28B gene and HCC.

The limitations of the present study were that viral factors associated with hepatocarcinogenesis were incompletely investigated. Ogata et al. [2003] reported that HCV-1b strains might be associated with HCC

on the basis of the secondary structure of the amino-terminal portion of the HCV NS3 protein. Giménez-Barcons et al. [2001] reported that high amino acid variability within the NS5A of HCV might be associated with HCC in patients with HCV-1b-related cirrhosis. In the present study, the clinical impact of other regions on hepatocarcinogenesis could not be investigated, except for aa 70 and 91 in the HCV core region. The results should also be interpreted with caution since patients infected with HCV-1a were not included. Other limitations include lack of analysis of the effects of life-style related diseases (such as diabetes, insulin resistance or non-alcoholic steatohepatitis) on hepatocarcinogenesis, except for fasting plasma glucose and total cholesterol [Sumida et al., 2010a,b]. The impact of viral factors and life-style related diseases on hepatocarcinogenesis should also be investigated in future studies.

In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of hepatocarcinogenesis following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy.

REFERENCES

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

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. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 46:1357–1364.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. 2010. Amino acid substitution in HCV core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 52:421–429.

Bruno S, Crosignani A, Maisonneuve P, Rossi S, Silini E, Mondelli MU. 2007. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: A seventeen-year prospective cohort study. Hepatology 46: 1350–1356.

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.

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

Donlin MJ, Cannon NA, Yao E, Li J, Wahed A, Taylor MW, Belle SH, Di Bisceglie AM, Aurora R, Tavis JE. 2007. Pretreatment sequence diversity differences in the full-length hepatitis C virus open reading frame correlate with early response to therapy. J Virol 81:8211–8224.

Fishman SL, Factor SH, Balestrieri C, Fan X, Dibisceglie AM, Desai SM, Benson G, Branch AD. 2009. Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. Clin Cancer Res 15:3205–3213.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Giménez-Barcons M, Franco S, Suárez Y, Forns X, Ampurdanès S, Puig-Basagoiti F, Sánchez-Fueyo A, Barrera JM, Llovet JM, Bruix J, Sánchez-Tapias JM, Rodés J, Saiz JC. 2001. High amino acid variability within the NS5A of hepatitis C virus (HCV) is associated with hepatocellular carcinoma in patients with HCV-1b-related cirrhosis. Hepatology 34:158–167.

Hézode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourlière M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM. Zeuzem S, PROVE2 Study Team. 2009. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 360:1839–1850.

Hirakawa M, Ikeda K, Arase Y, Kawamura Y, Yatsuji H, Hosaka T, Sezaki H, Akuta N, Kobayashi M, Saitoh S, Suzuki F, Suzuki Y, Kumada H. 2008. Hepatocarcinogenesis following HCV RNA eradication by interferon in chronic hepatitis patients. Intern Med 47:1637–1643.

Hu Z, Muroyama R, Kowatari N, Chang J, Omata M, Kato N. 2009. Characteristic mutations in hepatitis C virus core gene related to the occurrence of hepatocellular carcinoma. Cancer Sci 100: 2465–2468.

Ikeda K, Kobayashi M, Saitoh S, Someya T, Hosaka T, Akuta N, Suzuki F, Tsubota A, Suzuki Y, Arase Y, Kumada H. 2003. Recurrence rate and prognosis of patients with hepatocellular carcinoma that developed after elimination of hepatitis C virus RNA by interferon therapy. A closed cohort study including matched control patients. Oncology 65:204–210.

Ikeda M, Fujiyama S, Tanaka M, Sata M, Ide T, Yatsuhashi H, Watanabe H. 2005. Risk factors for development of hepatocellular carcinoma in patients with chronic hepatitis C after sustained response to interferon. J Gastroenterol 40:148–156.

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

Kobayashi S, Takeda T, Enomoto M, Tamori A, Kawada N, Habu D, Sakaguchi H, Kuroda T, Kioka K, Kim SR, Kanno T, Ueda T, Hirano M, Fujimoto S, Jomura H, Nishiguchi S, Seki S. 2007. Development of hepatocellular carcinoma in patients with chronic hepatitis C who had a sustained virological response to interferon therapy: A multicenter, retrospective cohort study of 1124 patients. Liver Int 27:186–191.

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

McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ, PROVE1 Study Team. 2009. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 360:1827–1838.

McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM, PROVE3 Study Team. 2010. Telaprevir for previously treated chronic HCV infection. N Engl J Med 362:1292–1303.

Montes-Cano MA, García-Lozano JR, Abad-Molina C, Romero-Gómez M, Barroso N, Aguilar-Reina J, Núñez-Roldán A, González-Escribano MF. 2010. Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. Hepatology 52:33–37.

Moriishi K, Okabayashi T, Nakai K, Moriya K, Koike K, Murata S, Chiba T, Tanaka K, Suzuki R, Suzuki T, Miyamura T, Matsuura Y. 2003. Proteasome activator PA28gamma-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 PA28gamma 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.

Nakamoto S, Imazeki F, Fukai K, Fujiwara K, Arai M, Kanda T, Yonemitsu Y, Yokosuka O. 2010. Association between mutations in the core region of hepatitis C virus genotype 1 and hepatocellular carcinoma development. J Hepatol 52:72–78.

Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T. 1999. Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? J Hepatol 30:1014–1022.

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.

Ogata S, Florese RH, Nagano-Fujii M, Hidajat R, Deng L, Ku Y, Yoon S, Saito T, Kawata S, Hotta H. 2003. Identification of hepatitis C virus (HCV) subtype 1b strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein. J Clin Microbiol 41:2835–2841.

Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY. Swiss Hepatitis C Cohort Study; Swiss HIV Cohort Study. 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: A genome-wide association study. Gastroenterology 138:1338–1345.

Sumida Y, Eguchi Y, Ono M. 2010a. Current status and agenda in the diagnosis of nonalcoholic steatohepatitis in Japan. World J Hepatol 2:374–383.

Sumida Y, Kanemasa K, Hara T, Inada Y, Sakai K, Imai S, Yoshida N, Yasui K, Itoh Y, Okanoue T, Yoshikawa T. 2010b. Impact of amino acid substitutions in the hepatitis C virus genotype 1b core region on liver steatosis and glucose tolerance in noncirrhotic patients without overt diabetes. J Gastroenterol Hepatol [Epub ahead of print].

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1104.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Tokita H, Fukui H, Tanaka A, Kamitsukasa H, Yagura M, Harada H, Okamoto H. 2005. Risk factors for the development of hepatocellular carcinoma among patients with chronic hepatitis C who achieved a sustained virological response to interferon therapy. J Gastroenterol Hepatol 20:752–758.

Tsubota A, Arase Y, Someya T, Suzuki Y, Suzuki F, Saitoh S, Ikeda K, Akuta N, Hosaka T, Kobayashi M, Kumada H. 2005. Early viral kinetics and treatment outcome in combination of high-dose interferon induction vs. pegylated interferon plus ribavirin for naive patients infected with hepatitis C virus of genotype 1b and high viral load. J Med Virol 75:27–34.

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Original Article

Efficacy and safety in sitagliptin therapy for diabetes complicated by chronic liver disease caused by hepatitis C virus

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Aim: Diabetes is present in patients with chronic liver disease caused by hepatitis C virus (HCV). The aim of this case–control study is to assess the efficacy and safety of dipeptidyl peptidase-4 inhibitor (sitagliptin) for type 2 diabetes mellitus (T2DM) with chronic liver disease caused by HCV.

Methods: Sixteen HCV positive patients with T2DM treated by sitagliptin were retrospectively enrolled. These patients were given sitagliptin between December 2009 and January 2010. Another 16 HCV patients with T2DM treated only with diet and excise for 48 weeks were selected as the control group. Serum levels of fasting plasma glucose (FPG), hemoglobin A1C (HbA1C), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured before and 12, 24, 36 and 48 weeks after the initiation of treatment.

Results: In the sitagliptin group, the average HbA1C level decreased approximately 0.8% at 48 weeks after the initiation of sitagliptin. Next, the average FPG level decreased approximately 20 mg/dL during follow up after the initiation of sitagliptin. All the patients were able to take sitagliptin of 50 mg/day without reduction because of sitagliptin-related side-effects. On the other hand, in the control group, the average HbA1C and FPG level did not change with statistical significance during follow up of 48 weeks. Regarding aminotransferase, there were no significant changes of average AST and ALT level during follow up of 48 weeks in both the sitagliptin group and control group.

Conclusion: Our results indicate that sitagliptin is effective and safe for the treatment of T2DM complicated with HCV positive chronic liver disease.

Key words: hepatitis C virus, sitagliptin, type 2 diabetes mellitus

INTRODUCTION

HEPATITIS C VIRUS (HCV) is one of the more common causes of chronic liver disease in the world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20–50% of cases over a period of 10–30 years.[1,2] In addition, HCV is a major risk for hepatocellular carcinoma (HCC).[3–7] Lately, it has been reported that chronic HCV infection is associated with type 2 diabetes mellitus (T2DM)[8–14] Moreover, T2DM has been suggested to enhance with the development of HCC and poor prognosis of liver transplantation.[15–19] Thus, in patients with chronic liver diseases, the management of T2DM is very important to improve the prolonged prognosis.

However, most oral hypoglycemic agents are metabolized in the liver and often induce the liver damage. Thus, it is difficult to treat the patients who have T2DM complicated with chronic liver disease.[20] A new oral hypoglycemic agent, dipeptidyl peptidase-4 (DPP-4)

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