GENETIC POLYMORPHISM-DISEASE ASSOCIATION

HLA-DP gene polymorphisms and hepatitis B infection in the Japanese population

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The mechanisms underlying the different outcomes of hepatitis B virus (HBV) infection are not fully understood.[1] Kamatani et al[2] identified an association of the single nucleotide polymorphisms (SNPs) human leukocyte antigen (*HLA*)-*DPA1* (rs3077) and *HLA-DPB1* (rs9277535) with chronic HBV infection in a genome-wide association study (GWAS). Additional studies confirmed that rs3077 and rs9277535 were associated with chronic HBV infection in the Han-Chinese population and strengthened the findings from previous GWAS.[3-6] Furthermore, Hu et al[7] reported that SNPs in *HLA-DP* (rs3077 and rs9277535) were associated with both HBV clearance and hepatocellular carcinoma (HCC) development. To investigate the association of these *HLA-DP* variants with the disease progression of HBV infection, we genotyped the 2 SNPs (rs3077 and rs9277535) in different clinical stages of liver disease in Japanese HBV carriers.

CLINICAL SUMMARY

A total of 241 HBV carriers (positive for hepatitis B surface antigen) who visited the clinics for liver diseases at the Nagasaki University Hospital or Nagasaki Medical Center between 1999 and 2007 were enrolled. As controls, 143 healthy Japanese volunteers (56 men and 87 women aged 16–63 years, with a mean age of 31.3 ± 8.9 years) without any history of liver disease were enrolled. All patients did not have any other types of liver diseases, such as chronic hepatitis C, alcoholic liver disease, autoimmune liver disease, or metabolic liver disease. The study protocol was approved by the Ethics Committees of National Nagasaki Medical Center, and informed consent was obtained from each individual. Of the 241 HBV carriers, 69 were considered to be asymptomatic carriers on the basis of sustained normalization of the serum alanine aminotransferase (ALT) levels together with seropositivity for anti-hepatitis Be antigen throughout the study. On the other hand, 172 of the 241 HBV carriers were considered to have chronic liver disease, such as chronic hepatitis (57), cirrhosis (65), or HCC (50) manifested by elevated ALT levels and by clinical or histologic findings on examination of liver tissue during the follow-up period. Of the 50 patients with HCC, 6 (12%) were found to have chronic hepatitis and 44 (88%) had cirrhosis. All patients were regularly followed with measurements of serum ALT and HBV markers, such as hepatitis B surface antigen, hepatitis Be antigen, anti-hepatitis Be antibody, and HBV-DNA. A total of 79 patients had undergone liver biopsy during the study to assess the degree of liver fibrosis. However, liver biopsy was not performed in patients who had apparent biochemical, endoscopic, and ultrasound features of liver cancer. Tumor markers such as alpha-fetoprotein and des-γ-carboxy-prothrombin were measured with ultrasonography of the liver every 6 months to detect HCC in an early stage. The diagnosis of HCC was made by several imaging modalities in all patients and confirmed histologically by sonography-guided fine-needle tumor biopsy specimens. The genotype of rs3077 (*HLA-DPA1*) and rs9277535 (*HLA-DPB1*) was determined by direct sequencing. The apolipoprotein B mRNA-editing enzyme catalytic peptide 3G (*APOBEC3G* H186R) genotyping was performed on the basis of the report by An et al.[8]

The frequencies of the 2 SNPs of *HLA-DPA1* (rs3077) and *HLA-DPB1* (rs9277535) are listed in Table I. There was a significant difference in the frequencies between these 2 SNPs between Japanese HBV carriers and healthy subjects, as described previously.[3] We divided HBV carriers into 2 groups: a nonadvanced group (asymptomatic carriers or chronic hepatitis, n = 115) and an advanced group (liver cirrhosis or HCC, n = 126). The frequencies of CC (rs3077) or GG (rs9277535) genotypes were higher in the advanced group compared with those in the nonadvanced group; however, the difference was not significant (Table I). Next, we stratified the HBV carriers for the presence or absence of the *APOBEC3G* H186R variant and examined the effects of *HLA-DP* polymorphisms on the progression of HBV-related liver disease. Both C and G alleles of rs3077 and rs9277535 significantly increased the risk for advanced liver disease in HBV carriers lacking the H186R variant (Table II).

A 2-stage GWAS identified SNPs including rs3077 and rs9277535 located in *HLA-DPA1* and *HLA-DPB1*, which were associated with a susceptibility to chronic HBV infection.[2] After the first Japanese GWAS, 5 studies replicated the association of these 2 *HLA-DP* SNPs (rs3077 and rs9277535) and chronic HBV infection in the Han-Chinese population.[3-7] Among these studies, an association between HBV-related HCC and rs9277535 or rs3077 was demonstrated.[7] In this study, we examined whether these 2 SNPs (rs3077 and rs9277535) in *HLA-DP* genes were associated with the disease progression and susceptibility to HBV infection in a Japanese population. As demonstrated previously, we reconfirmed that rs3077 and rs9277535 in the *HLA-DPA1* and *HLA-DPB1* genes were significantly associated with HBV infection. Although some differences in the frequencies of rs3077 and rs9277535 genotypes between HBV carriers with advanced liver disease (liver cirrhosis and HCC) and those without advanced liver disease were observed, these differences were not statistically significant.

Recent evidence suggests that APOBEC3G inhibits HBV production by interfering with HBV replication through hypermutation of the majority of the HBV genome.[8] Because of the *APOBEC3G* gene's ability to regulate HBV replication, mutations of the gene may cause a deleterious variation that may affect the outcome of HBV infection. Among the SNPs identified in the *APOBEC3G* gene, H186R variant was strongly associated with a decline in $CD4^+$ T-cell numbers and accelerated progression to acquired immune deficiency syndrome–defining conditions in human immunodeficiency virus–infected individuals.[9,10] Viral disease outcome is influenced by host variability in immune response genes and genes that control viral replication or mutation rate.[11] *APOBEC3G* coding region variant might influence the progression of HBV infection by inducing the replication of HBV.[12] Therefore, genetic diversity of immune response genes, such as *HLA*, and genes that control viral replication, such as *APOBEC3G*, could contribute to the variability in outcome of HBV infection. To minimize the effects

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Conflict of interests: None

Funding: This study was supported by a grant from the National Hospital Organization (Liver-net work study).

Submitted for publication May 25, 2012; revision submitted June 8, 2012; accepted for publication June 9, 2012.

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1931-5244/$ - see front matter

http://dx.doi.org/10.1016/j.trsl.2012.06.003

Table I. Association between *HLA-DP* polymorphisms (rs3077, rs9277535) and HBV infection

SNP ID	HBV carrier n = 241 (%)	Healthy subjects n = 143 (%)	*P* value*	OR (95% CI)	Advanced HBV carrier n = 115 (%)	Nonadvanced HBV carrier n = 126 (%)	*P* value*	OR (95% CI)
rs3077								
C/C	148 (61.4)	47 (32.9)			77 (67.0)	71 (56.3)		
C/T	79 (32.8)	72 (50.3)			33 (28.7)	46 (36.5)		
T/T	14 (5.8)	24 (16.8)			5 (4.3)	9 (7.1)		
C allele (allele frequencies)	375 (77.8)	166 (58.0)	<0.0001	2.533 (1.843–3.483)	187 (81.3)	188 (74.6)	0.077	1.480 (0.957–2.290)
rs9277535								
G/G	143 (59.3)	45 (31.5)			73 (63.5)	70 (55.6)		
A/G	82 (34.0)	72 (50.3)			36 (31.3)	46 (36.5)		
A/A	16 (6.6)	26 (18.2)			6 (5.2)	10 (7.9)		
G allele (allele frequencies)	368 (76.3)	162 (56.6)	<0.0001	2.471 (1.804–3384)	182 (79.1)	186 (73.8)	0.170	1.345 (0.880–2.056)

Abbreviations: CI, confidence interval; *HBV,* hepatitis B virus; *OR,* odds ratio; *SNP,* single-nucleotide polymorphism.
**P* values were calculated using the chi-square test.

Table II. Association between *HLA-DP* polymorphisms (rs3077, rs9277535) and the outcome of HBV infection in HBV carrier without H186R variant

SNP ID	Advanced HBV carrier n = 90 (%)	Nonadvanced HBV carrier n = 108 (%)	*P* value*	OR (95% CI)
rs3077				
C/C	64 (71.1)	60 (55.6)		
C/T	22 (24.4)	40 (37.0)		
T/T	4 (4.4)	8 (7.4)		
C allele (allele frequencies)	150 (83.3)	160 (74.1)	0.026	1.750 (1.065–2.874)
rs9277535				
G/G	5 (5.6)	10 (9.3)		
A/G	24 (26.7)	39 (36.1)		
A/A	61 (67.8)	59 (54.6)		
G allele (allele frequencies)	146 (81.1)	157 (72.7)	0.049	1.614 (1.000–2.604)

Abbreviations: CI, confidence interval; *HBV,* hepatitis B virus; *OR,* odds ratio; *SNP,* single-nucleotide polymorphism.
**P* values were culculated using the chi-square test.

of viral factors, such as APOBEC3G-mediated HBV editing, and evaluate the effect of *HLA-DP* more precisely, we focused on the subjects without the H186R variant. Because the *APOBEC3G* coding region variant might influence the progression of HBV infection,[11] we investigated the effect of *HLA-DP* polymorphisms on the outcome of HBV infection in HBV carriers lacking the H186R variant.

Our results showed that *HLA-DP* polymorphisms were associated with the progression of HBV infection and that this association was significant in Japanese HBV carriers lacking H186R variants. Our data demonstrated that *HLA-DP* polymorphisms are important in determining the susceptibility and the progression of HBV infection in the Japanese population.

One limitation of our study is the lack of information of HBV genotypes in the patients studied. Another limitation is that the number of HBV carriers (n = 241) is relatively small. Larger studies are needed to confirm the results of our study.

CONCLUSIONS

We confirmed that rs3077 and rs9277535 SNPs in the *HLA-DP* locus are associated with the susceptibility and progression of HBV infection in the Japanese population. Further functional analyses are warranted to validate the biological plausibility of these SNPs in chronic HBV infection.

REFERENCES

1. Ganem D, Prince AM. Hepatitis B virus infection–natural history and clinical consequences. N Engl J Med 2004;350:1118–29.
2. Kamatani Y, Wattanapokayakit S, Ochi H, et al. A genome-wide association study identifies variants in the HLA-DP locus associated with chronic hepatitis B in Asians. Nat Genet 2009;41:591–5.
3. An P, Winkler C, Guan L, O'Brien SJ, Zeng Z. HBV Study Consortium. A common HLA-DPA1 variant is a major determinant of hepatitis B virus clearance in Han Chinese. J Infect Dis 2011;203:943–7.
4. Guo X, Zhang Y, Li J, et al. Strong influence of human leukocyte antigen (HLA)-DP gene variants on development of persistent chronic hepatitis B virus carriers in the Han Chinese population. Hepatology 2011;53:422–8.
5. Wang L, Wu XP, Zhang W, et al. Evaluation of genetic susceptibility loci for chronic hepatitis B in Chinese: two independent case-control studies. PLoS One 2011;6:e17608.
6. Li J, Yang D, He Y, et al. Associations of HLA-DP variants with hepatitis B virus infection in southern and northern Han Chinese populations: a multicenter case-control study. PLoS One 2011;6:e24221.
7. Hu L, Zhai X, Liu J, et al. Genetic variants in HLA-DP/DQ influence both hepatitis B virus clearance and hepatocellular carcinoma development. Hepatology 2012;55:1426–31.
8. An P, Bleiber G, Duggal P, et al. APOBEC3G genetic variants and their influence on the progression to AIDS. J Virol 2004;78:11070–6.
9. Suspène R, Guétard D, Henry M, Sommer P, Wain-Hobson S, Vartanian JP. Extensive editing of both hepatitis B virus DNA strands by APOBEC3 cytidine deaminases in vitro and in vivo. Proc Natl Acad Sci U S A 2005;102:8321–6.
10. Do H, Vasilescu A, Diop G, et al. Exhaustive genotyping of the CEM15 (APOBEC3G) gene and absence of association with AIDS progression in a French cohort. J Infect Dis 2005;191:159–63.
11. Martin MP, Carrington M. Immunogenetics of viral infections. Curr Opin Immunol 2005;17:510–6.
12. Bonvin M, Greeve J. Hepatitis B. modern concepts in pathogenesis–APOBEC3 cytidine deaminases as effectors in innate immunity against the hepatitis B virus. Curr Opin Infect Dis 2008;21:298–303.

Hepatology Research 2013 doi: 10.1111/hepr.12041

Original Article

Antitumor efficacy of transcatheter arterial chemoembolization with warmed miriplatin in hepatocellular carcinoma

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Aim: Patients with unresectable hepatocellular carcinoma (HCC) often undergo transcatheter arterial chemoembolization (TACE). Miriplatin is a lipophilic cisplatin derivative used in TACE that is effective in HCC. However, the difference in antitumor efficacy between warmed versus room temperature miriplatin is unclear.

Methods: Chemotherapy efficacy was evaluated by dynamic computed tomography 1–3 months after TACE, according to the Modified Response Evaluation Criteria in Solid Tumors. A total of 203 patients with HCC who received TACE with miriplatin for the first time were included in a follow-up study to retrospectively investigate its efficacy and safety. Overall, 45 patients underwent TACE with warmed (40°C) miriplatin and 158 patients received TACE with room temperature miriplatin.

Results: Seventy patients (44.3%) treated with room temperature miriplatin and 32 patients (71.1%) who received warmed miriplatin experienced complete or partial responses. Multivariate analysis identified miriplatin temperature (warmed miriplatin, risk ratio (RR) = 2.26, $P = 0.047$), tumor number (solitary, RR = 3.48, $P = 0.007$), α-fetoprotein (AFP) level (<50 ng/mL, RR = 2.35, $P = 0.012$) and history of TACE (no history, RR = 2.22, $P = 0.041$) as predictors of objective response following TACE with miriplatin, and no serious complications were observed.

Conclusion: Warm temperature, solitary tumors, low AFP level and first TACE are significant and independent predictors of objective response after TACE using miriplatin. These results suggest that warmed miriplatin can be considered as one of the standard treatments for unresectable HCC.

Key words: hepatocellular carcinoma, miriplatin, transcatheter arterial chemoembolization

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common malignant diseases worldwide.[1] In Japan, more than 30 000 people die of HCC each year, and HCC ranks third and fifth in men and women, respectively, as cause of death due to malignant neoplasms.[2] Because resection, liver transplantation and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation) are applicable in only 30–40% of HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC.[3–10] TACE is recommended for HCC patients with class A or B liver damage, two or three tumors, and a tumor diameter greater than 3 cm, according to the guidelines for treatment of HCC by the Japan Society of Hepatology in 2009.[11] The Barcelona Clinic Liver Cancer group recommends TACE for HCC patients with stage B and class A or B disease and more than four tumors, or stage C disease without portal vein invasion or extrahepatic metastasis.[12] Miriplatin (cis-[1R,2R]-1,2-cyclohexanediamine-N,N′]bis[myristate])-platinum(II) monohydrate; Dainippon Sumitomo Pharma, Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent.[13–16] Some trials reported that miriplatin is effective for HCC.[17,18] Addition of embolizing agents to miriplatin-based treatment has been shown to result in a higher response in patients with

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Received 26 August 2012; revision 11 November 2012; accepted 3 December 2012.

HCC.[19] Significant predictors for complete response to miriplatin include solitary tumors, previous complete response to TACE via injection from the peripheral to segmental hepatic artery,[20] and stage I or II disease.[21] The most important issue regarding TACE with miriplatin is its viscosity: due to its high viscosity, miriplatin/lipiodol suspension cannot enter smaller vessels. We previously determined that warming miriplatin to 40°C decreased its viscosity in vitro (unpubl. obs.). We investigated the viscosity of miriplatin/lipiodol suspension using a viscometer (μVISC; RHEOSENSE, San Ramon, CA, USA). The miriplatin/lipiodol suspension was adjusted to 20 mg/mL, and then warmed to 40°C. We measured the viscosity of these solutions at room temperature and 40°C three times, and determined that the mean viscosity of miriplatin/lipiodol suspension at room temperature and 40°C is 37.48 mPa-S and 21.42 mPa-S, respectively. The purpose of this retrospective study was to evaluate the antitumor efficacy and adverse effects of TACE with warmed miriplatin suspension.

METHODS

Patients

A TOTAL OF 402 HCC Japanese adult patients were consecutively recruited into the study protocol of TACE with miriplatin from December 2007 to June 2012 at our center. Among them, 203 patients who received miriplatin for the first time and who were assessed 1–3 months after TACE were enrolled in this retrospective study. Warmed miriplatin was used for all patients from August 2011 to June 2012. Overall, 45 patients received warmed miriplatin and 158 patients received room temperature miriplatin.

Table 1 summarizes the profile and laboratory data of the study patients. The median follow-up period, from the end of TACE until the last visit, was 458 days (range, 57–1226 days). Higher serum aspartate aminotransferase (AST) levels and prothrombin activity were observed in patients in the room temperature miriplatin group compared to those in the warmed miriplatin group. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

HCC

Before treatment with miriplatin, all patients underwent a comprehensive evaluation consisting of a medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver-imaging studies (dynamic computed tomography [CT], ultrasonography [US], digital-subtraction angiography [DSA]), complete blood count and blood chemistry. Diagnosis of HCC was established based on the findings

Table 1 *Profile and pretreatment laboratory data of 203 patients who underwent TACE using miriplatin/lipiodol suspension under room temperature and warmed conditions for unresectable HCC*

	Total	Room temperature miriplatin group	Warmed miriplatin group	*P*-value
Demographic data				
No. of patients	203	158	45	
Sex (male/female)	130/73	99/59	31/14	0.485
Age, years†	73 (45–91)	71 (45–91)	74 (48–86)	0.940
Etiology, HBV/HCV/other	24/161/18	17/130/11	7/31/7	0.097
Laboratory data†				
Albumin, g/dL	3.0 (2.0–4.2)	3.3 (2.0–4.2)	3.0 (2–4.1)	0.553
Serum aspartate aminotransferase, IU/L	50 (18–415)	52 (18–415)	47 (19–305)	0.033
Serum alanine aminotransferase, IU/L	34 (12–282)	34 (12–171)	31 (12–282)	0.311
Total bilirubin, mg/dL	1.0 (0.4–4.9)	1.1 (0.4–4.9)	1.0 (0.4–2.7)	0.902
Platelet count, $\times 10^3/mm^3$	9.6 (1.9–28.2)	9.5 (1.9–28.2)	10.0 (3.5–26.5)	0.716
Prothrombin activity, %	79.2 (40.8–123.1)	81.5 (45.7–123.1)	74.0 (40.8–106.1)	0.005
AFP, μg/L	30.0 (1.8–282 200)	32.3 (1.8–282 200)	22.0 (2.9–49 710)	0.527
AFP-L3, %	19.0 (0–82.7)	22.7 (0–82.7)	12.0 (0–78.0)	0.601
DCP, AU/L	39.0 (4–662 000)	40.5 (4–65 290)	30 (8–662 000)	0.748
Child–Pugh class, A/B	152/51	119/39	33/12	0.846

Data are shown as number and percentage of patients, except those denoted by †, which represent the median (range) values.
AFP, α-fetoprotein; AFP-L3, *Lens culinaris* agglutinin-reactive fraction of AFP; DCP, des-γ-carboxy prothrombin; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; TACE, transcatheter arterial chemoembolization.

of dynamic CT, US and DSA. Patients who had extrahepatic metastasis of HCC or other malignancies were excluded.

Table 2 summarizes the tumor profiles and TACE treatment history of patients in each study group. In the warmed miriplatin group, 12 patients (26.7%) had a solitary tumor and 33 patients (73.3%) had multiple tumors. The median diameter of the largest tumor was 30 mm (range, 6–115 mm) and 29 patients (64.4%) had a history of TACE. In the room temperature miriplatin group, 29 patients (18.4%) had a solitary tumor and 129 patients (81.6%) had multiple tumors. The median diameter of the largest tumor was 30 mm (range, 6–125 mm), and 120 patients (75.9%) had a history of TACE. Patients in the room temperature miriplatin group tended to have more tumors than those in the warmed miriplatin group.

Treatment protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and a 4-Fr Shepherd Hook catheter (FansacIV or Angiomaster; Terumo Clinical Supply, Gifu, Japan) was inserted into the hepatic artery, and portography through the superior mesenteric artery and celiac arteriography were performed. Then, a 2.0- or 2.1-Fr microcatheter was advanced into the feeding arteries of each tumor, and miriplatin suspended in lipiodol solution was injected into the hepatic artery; however, the injection was discontinued immediately before the flow ceased completely. Thereafter, the feeding arteries to the tumors were embolized with 1-mm gelatin cubes (Gelpart; Nippon Kayaku, Tokyo, Japan). The miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was 120–180 mg/2–3 mL and was determined based on tumor size and degree of liver dysfunction. A 5-HT3 antagonist was administrated before the miriplatin injection; however, hydration by i.v. fluid administration was not conducted before the TACE procedure. A clean container was placed in an electric range filled with water. The injector of miriplatin/lipiodol suspension and sterilized physiological saline were then placed in the container, and the container was warmed to 60°C. We observed that in 60°C water, the miriplatin/lipiodol suspension in the injector reaches 40°C *in vitro*. The stability of warmed miriplatin/lipiodol suspension has been previously reported.

Assessment of therapeutic efficacy

The efficacy of chemotherapy was evaluated by dynamic CT 1–3 months after TACE with miriplatin, and was based on change in the maximum diameter of viable target lesions (i.e. those showing enhancement in the arterial phase). Response categories, according to the Modified Response Evaluation Criteria in Solid Tumors[22] are as follows: complete response (CR), disappearance of any intratumoral arterial enhancement in all target lesions; partial response (PR), at least a 30% decrease in the sum of diameters of viable target lesions; stable disease (SD), any cases that do not qualify for either PR or progressive disease; and progressive disease (PD), an increase of at least 20% in the sum of the diameters of viable target lesions.

Toxicity evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria (ver. 4.0). Within 2 weeks before TACE with miriplatin, and at 3–7 days (three times during this period) and at 1 month afterward, hematological (i.e. leukocyte

Table 2 Tumor profile and treatment history of 203 patients who underwent TACE using miriplatin/lipiodol suspension under room temperature condition and warmed conditions for unresectable HCC

	Total	Room temperature miriplatin group	Warmed miriplatin group	*P*-value
No. of patients	203	158	45	
Tumor size, mm†	20 (6–125)	30 (6–125)	30 (6–115)	0.435
Tumor multiplicity (solitary/multiple)	41/162	29/129	12/33	0.291
No. of tumors†	3 (1–100)	3 (1–100)	3 (1–40)	0.030
Stage (I/II/III/IV)	54/81/66/2	38/67/51/2	16/14/15/0	0.329
History of TACE	73.4%	75.9%	64.4%	0.130

Data are shown as number and percentage of patients, except those denoted by †, which represent the median (range) values.
HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

and thrombocyte counts) and clinical chemistry (i.e. serum AST, serum alanine aminotransferase [ALT], albumin, total bilirubin, serum creatine and prothrombin activity) toxicity evaluations were conducted.

Statistical analysis

The distribution of subject characteristics was assessed by the χ^2-test or Mann–Whitney *U*-test, as appropriate. Logistic analysis was used to determine independent predictive factors associated with CR and PR by TACE with miriplatin. The risk ratio (RR) and 95% confidence interval (CI) were also calculated. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using SPSS software (SPSS, Chicago, IL, USA). All *P*-values of less than 0.05 by two-tailed test were considered significant.

RESULTS

Treatment effects

OF THE 203 treated patients, 55 (27.1%) experienced a CR, 47 patients (23.2%) PR, 66 patients (32.5%) SD and 33 patients (17.2%) PD. Overall, 50.3% of patients achieved an objective response (i.e. CR plus PR).

Predictive factors associated with objective response to TACE

Data from the entire study population were analyzed to identify factors that could predict objective response. Univariate analysis identified five parameters that tended to correlate or significantly correlated with objective response: miriplatin temperature (warmed miriplatin, $P = 0.002$), tumor number (solitary tumor, $P < 0.001$), α-fetoprotein (AFP) level (<50 ng/mL, $P = 0.003$), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%) (<10%, $P = 0.032$) and history of TACE (no history, $P = 0.002$). These five factors were entered into multivariate analysis, which revealed four parameters to be significant and independent determinants of objective response using miriplatin: miriplatin temperature (warmed miriplatin, risk ratio [RR] = 2.26, $P = 0.047$), tumor number (solitary tumor, RR = 3.48, $P = 0.007$), AFP level (<50 ng/mL, RR = 2.35, $P = 0.012$) and history of TACE (no history, RR = 2.22, $P = 0.041$) (Table 3).

Objective response according to AFP-L3%

Patients were divided into two groups according to AFP-L3 serum level using a cut-off value of 10% (low AFP-L3 group [<10%], $n = 83$; high AFP-L3 group [≥10%], $n = 89$). In the high AFP-L3 group, 27 of 83 patients (32.5%) experienced CR, 22 patients (26.5%) PR, 26 patients (31.3%) SD and eight patients (9.6%) PD. In the low AFP-L3 group, 17 of 89 patients (19.1%) experienced CR, 20 patients (22.5%) PR, 29 patients (32.6%) SD and 23 patients (25.8%) PD. The response rates were significantly different between the two groups ($P = 0.032$, log-rank test).

Objective response according to miriplatin temperature, tumor number, AFP and history of TACE

Next, the efficacy of TACE using miriplatin according to temperature condition was examined (Fig. 1). In the warmed miriplatin group, 19 of 45 patients (42.2%) experienced CR, 13 patients (28.9%) PR, eight patients (17.8%) SD and five patients (11.1%) PD. In the room temperature miriplatin group, 36 of 158 patients

Table 3 Factors associated with objective response (CR plus PR) after TACE using miriplatin, identified by multivariate analysis

Factors	Category	Risk ratio (95% confidence interval)	*P*-value†
Miriplatin condition	1: Room temperature	1	0.047
	2: Warmed	2.26 (1.01–5.04)	
Tumor number	1: Multiple nodules	1	0.007
	2: Solitary nodule	3.48 (1.42–8.62)	
AFP	1: <50 ng/mL	1	0.012
	2: ≥50 ng/mL	2.35 (1.21–4.57)	
History of TACE	1: Yes	1	0.041
	2: No	2.22 (1.03–4.75)	

†Cox proportional hazard model.
AFP, α-fetoprotein; CR, complete response; PR, partial response; TACE, transcatheter arterial chemoembolization.

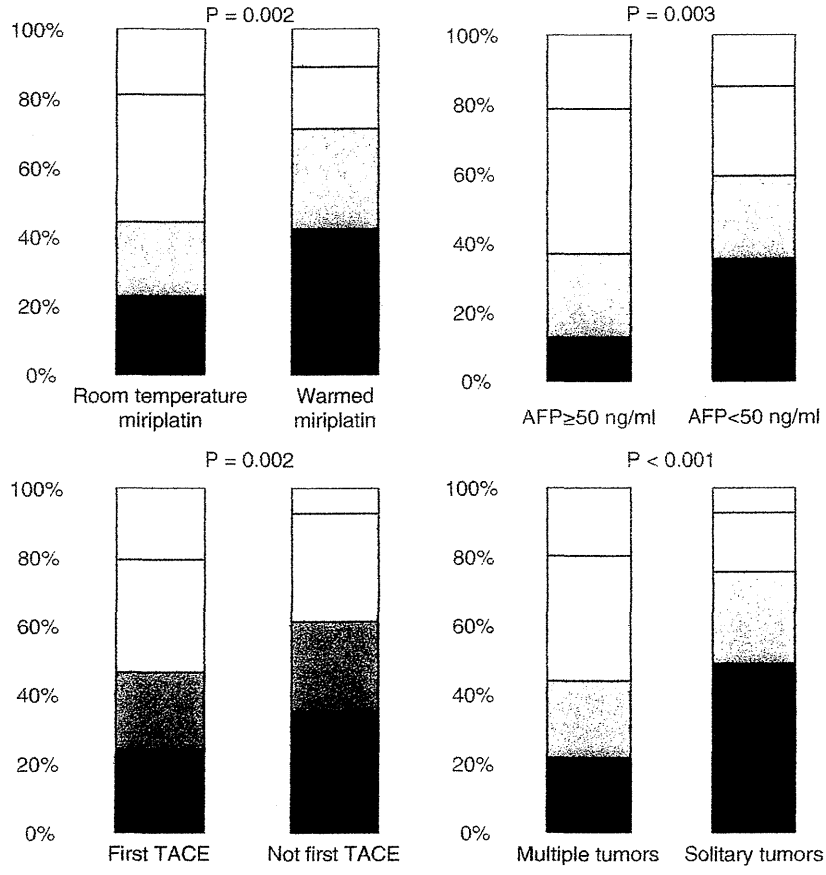

Figure 1 Efficacy of transcatheter arterial chemoembolization (TACE) using miriplatin in patients with hepatocellular carcinoma according to miriplatin temperature, serum α-fetoprotein (AFP) level, history of TACE and tumor number. Complete response (CR) and partial response (PR) rates were significantly higher for patients who received warmed miriplatin, had a low AFP level, were undergoing their first TACE and/or had solitary tumors. □, progressive disease (PD); ▨, stable disease (SD); ▩, PR; ■, CR.

(22.8%) experienced CR, 34 patients (21.5%) PR, 58 patients (36.7%) SD and 30 patients (19.0%) PD. Overall, 71.1% of patients in the warmed miriplatin group and 44.3% of patients in the room temperature miriplatin group experienced an objective response (i.e. CR plus PR). The rates were significantly different between the two groups ($P = 0.002$, log–rank test).

In the high AFP group (≥50 ng/mL, $n = 79$), 10 of 79 patients (12.7%) experienced CR, 19 patients (24.1%) PR, 33 patients (41.8%) SD and 17 patients (21.5%) PD. In the low AFP group (<50 ng/mL, $n = 113$), 40 of 113 patients (35.4%) experienced CR, 27 patients (23.9%) PR, 29 patients (25.7%) SD and 17 patients (15.0%) PD (Fig. 1). The rates were significantly different between the two groups ($P = 0.003$, log–rank test).

In the TACE-naïve group ($n = 54$), 19 of 54 patients (35.2%) experienced CR, 14 patients (25.9%) PR, 17 patients (31.5%) SD and four patients (7.4%) PD. In patients who had previously undergone TACE ($n = 149$), 36 of 149 patients (24.2%) experienced CR, 33 patients (22.1%) PR, 49 patients (32.9%) SD and 31 patients (20.8%) PD (Fig. 1). The rates were significantly different between the two groups ($P = 0.002$, log–rank test).

Among all patients, 41 patients (20.2%) had a solitary tumor and 162 (79.8%) had multiple tumors. In the solitary tumor group, 20 of 41 treated patients (48.8%) experienced CR, 11 patients (26.8%) PR, seven patients (17.1%) SD and three patients (7.3%) PD. In the multiple tumors group, 35 of 162 patients (21.6%) experienced CR, 36 patients (22.2%) PR, 59 patients (36.4%) SD and 32 patients (19.8%) PD (Fig. 1). The rates were significantly different between the two groups ($P < 0.001$, log–rank test).

Adverse effects

Fever, anorexia and elevated serum transaminase levels were observed in most patients after miriplatin administration (Table 4). In the room temperature miriplatin group and warmed miriplatin groups, the following grade 4 events were observed: increased AST in four (2.5%) and one patient (3.5%), respectively, and

Table 4 Adverse effects following miriplatin administration

	Room temperature condition ($n = 158$)				Warmed condition ($n = 45$)			
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
White blood cells decreased	11 (7.0%)	19 (12.0%)	1 (0.6%)	0	5 (10.7%)	4 (8.9%)	0	0
Anemia	96 (60.8%)	19 (12.0%)	5 (3.2%)	0	21 (46.7%)	6 (17.9%)	0	0
Platelet count decreased	80 (50.6%)	38 (24.1%)	20 (12.7%)	0	22 (48.9%)	10 (22.2%)	3 (6.7%)	0
Aspartate aminotransferase increased	75 (47.5%)	33 (20.9%)	38 (24.1%)	4 (2.5%)	21 (46.7%)	7 (15.6%)	20 (28.6%)	2 (4.4%)
Alanine aminotransferase increased	74 (46.8%)	17 (10.8%)	22 (13.9%)	1 (0.6%)	21 (46.7%)	10 (22.2%)	4 (8.9%)	2 (4.4%)
Fever	72 (45.6%)	17 (10.8%)	0	0	22 (48.9%)	7 (17.9%)	0	0
Appetite loss	63 (39.9%)	2 (1.3%)	0	0	25 (55.6%)	0	0	0
Abdominal pain	30 (0.6%)	5 (3.2%)	0	0	4 (10.7%)	2 (4.4%)	0	0

Values denote numbers of subjects. Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria ver. 4.0.

increased ALT in one (0.6%) and one patient (3.6%), respectively; all of these elevations resolved within 2 weeks. No vascular complications of the hepatic artery were observed in any patient. No other serious complications or treatment-related deaths were observed following miriplatin administration. No significant differences in adverse effects were observed between the two groups.

DISCUSSION

TRANSCATHETER ARTERIAL CHEMOEMBOLIZATION is widely performed in patients with HCC who are not eligible for curative therapy. Previous randomized controlled trials and meta-analyses confirmed the survival benefit of TACE. Because many anticancer drugs, such as doxorubicin, epirubicin, mitomycin C, cisplatin and neocarzinostatin, have been used for the treatment of HCC, the most effective and least toxic agents or protocol remain unclear.[23,24] In most patients, TACE can be repeated, and using the same agent multiple times can lead to resistance. A previous study reported that platinum analogs are frequently effective for advanced HCC that are unresponsive to TACE with epirubicin.[25] Miriplatin was developed as a lipophilic platinum complex that has superior antitumor efficacy in HCC with lower toxicity compared to cisplatin.[13–16] Previous reports suggested that TACE with miriplatin can be used safely for HCC patients with chronic renal failure.[26]

Pharmacokinetic studies have demonstrated that the plasma concentration of total platinum is much lower in patients treated with miriplatin compared with that in patients treated with intra-arterial cisplatin: the C_{max} is approximately 300-fold lower and the T_{max} roughly 500-fold longer for miriplatin than the corresponding values for intra-arterial cisplatin. Miriplatin/lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miriplatin. Miriplatin/lipiodol releases 1,2-diaminocyclohexane platinum (II) dichloride (DPC) as its active platinum compound, which binds to nuclear DNA and mediates miriplatin/lipiodol cytotoxicity. In a cisplatin-resistant rat hepatoma cell line model, cross-resistance to DPC was not observed.[27]

Previous studies reported the efficacy of miriplatin, but differences in efficacy associated with miriplatin temperature have not yet been evaluated. In the present study, we examined predictors of objective response to TACE with miriplatin. Multivariate analysis identified use of warmed miriplatin, low serum AFP, first TACE

and solitary tumors as predictors of objective response in patients who received TACE with miriplatin. Previous reports identified CR after previous TACE, solitary tumor, injection from peripheral to segmental hepatic artery,[20] and stage I or II disease[21] as significant predictors associated with CR to TACE with miriplatin. Another report stated that the rates of local recurrence and intratumoral recurrence in patients treated with epirubicin were significantly lower than those in patients treated with miriplatin.[28] In the present study, some of the above factors were not identified as significant predictors of response. The differences in the findings of the present study and the reports described above are not currently clear, but may reflect differences in the population samples, as this was the first study to focus on the objective response of patients receiving miriplatin for the first time. Notably, the present study is the first study to investigate the viscosity of miriplatin/lipiodol suspension. Further studies of larger populations including individuals of other ethnicities are necessary.

In this study, warmed miriplatin was associated with objective response after TACE. The main issue associated with miriplatin administration is its high viscosity, which prevents the miriplatin/lipiodol suspension from flowing into the peripheral artery and leads to inhomogeneous distribution of miriplatin/lipiodol suspension in HCC tumors. This is the primary reason that TACE with miriplatin is associated with reduced efficacy compared to TACE with other agents.[28] Basic research has provided evidence that as the temperature of miriplatin/lipiodol suspension rises, its viscosity decreases; for example, the viscosity of miriplatin/lipiodol suspension at 40°C is 0.51-times that at 25°C. The chemical behavior of miriplatin does not change until its temperature reaches 70°C. Further studies should be performed to investigate the viscosity and antitumor efficacy of condensed and warmed miriplatin conditions, as well as the associated wash-out periods. In addition, although no significant differences in adverse effects between groups were noted, further follow up regarding vascular complications of the hepatic artery is required.

Previous studies reported the relationship between tumor multiplicity and efficacy of TACE.[20] TACE can be performed selectively, and the dose of drug per tumor is higher in patients with solitary tumors than in those with multiple tumors. In the present study, solitary tumors and warmed miriplatin were associated with objective response. These results are not inconsistent with previous studies. Interestingly, in the present patients, the impact of warmed miriplatin and solitary tumor was more significant than that of age, liver function, tumor size, tumor stage, tumor markers, injection artery and history of TACE. One possible explanation for this finding is that the study population included patients who received TACE with miriplatin for the first time. Previous studies reported that complete tumor necrosis after TACE offered favorable long-term survival outcomes in HCC patients.[5,29] In the current study, warmed miriplatin administration was associated with objective response, suggesting that warmed miriplatin administration potentially results in a favorable prognosis for HCC.

The present study has certain limitations. This was a retrospective study and the patients were not randomized with respect to treatment with warmed versus room temperature miriplatin. A prospective study is needed to assess the safety and efficacy of warmed miriplatin administration. The other limitation is the small number of cases in the warmed miriplatin group. A study with a larger number of patients is required to confirm the present results. Furthermore, evaluation of the efficacy of warmed miriplatin compared with epirubicin or cisplatin in HCC is also required.

In conclusion, the present study identified warmed miriplatin and solitary tumors as significant and independent predictors of objective response after TACE using miriplatin. The results emphasize the importance of the condition under which miriplatin is administrated, and we recommend that warmed miriplatin should be the standard method of administration for patients with unresectable HCC undergoing TACE.

ACKNOWLEDGMENTS

THIS STUDY WAS supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

REFERENCES

1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55: 74–108.
2 Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. *J Gastroenterol* 2009; 44 (Suppl): 102–7.
3 Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S. Hepatic artery embolization in 120 patients with unresectable hepatoma. *Radiology* 1983; 148: 397–401.
4 Lin DY, Liaw YF, Lee TY, Lai CM. Hepatic arterial embolization in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. *Gastroenterology* 1988; 94: 453–6.

5 Ikeda K, Kumada H, Saitoh S, Arase Y, Chayama K. Effect of repeated transcatheter arterial embolization on the survival time in patients with hepatocellular carcinoma. *Cancer* 1991; **68**: 2150–4.
6 Llovet JM, Real MI, Montana X *et al.*; Barcelona Liver Cancer Group. Arterial embolisation or chemoembolization versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734–9.
7 Lo CM, Ngan H, Tso WK *et al.* Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164–71.
8 Camma C, Schepis F, Orlando A *et al.* Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. *Radiology* 2002; **224**: 47–54.
9 Ikeda M, Maeda S, Shibata J *et al.* Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. *Oncology* 2004; **66**: 24–31.
10 Takayasu K, Arii S, Ikai I *et al.*; Liver Cancer Study Group of Japan. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; **131**: 461–9.
11 Arii S, Sata M, Sakamoto M *et al.* Management of hepatocellular carcinoma: report of consensus meeting in the 45th annual meeting of the Japan Society of Hepatology (2009). *Hepatol Res* 2010; **40**: 667–85.
12 Bruix J, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. *Gastroenterology* 2004; **127** (5 Suppl 1): S179–S188.
13 Maeda M, Uchida NA, Sasaki T. Liposoluble platinum (II) complexes with antitumor activity. *Jpn J Cancer Res* 1986; **77**: 523–5.
14 Kishimoto S, Ohtani A, Fukuda H, Fukushima S, Takeuchi Y. Relation between intracellular accumulation and cytotoxic activity of cis-[((1R,2R)-1, 2-cyclohexanediamine- N, N')bis(myristato)]platinum(II) suspended in Lipiodol. *Biol Pharm Bull* 2003; **26**: 683–6.
15 Hanada M, Baba A, Tsutsumishita Y, Noguchi T, Yamaoka T. Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of human hepatoma cells orthotopically implanted in nude rats. *Cancer Sci* 2009; **100**: 189–94.
16 Hanada M, Baba A, Tsutsumishita Y *et al.* Intra-hepatic arterial administration with miriplatin suspended in an oily lymphographic agent inhibits the growth of tumors implanted in rat livers by inducing platinum-DNA adducts to form and massive apoptosis. *Cancer Chemother Pharmacol* 2009; **64**: 473–83.
17 Fujiyama S, Shibata J, Maeda S *et al.* Phase I clinical study of a novel lipophilic platinum complex (SM-11355) in patients with hepatocellular carcinoma refractory to cisplatin/lipiodol. *Br J Cancer* 2003; **89**: 1614–19.
18 Okusaka T, Okada S, Nakanishi T, Fujiyama S, Kubo Y. Phase II trial of intra-arterial chemotherapy using a novel lipophilic platinum derivative (SM-11355) in patients with hepatocellular carcinoma. *Invest New Drugs* 2004; **22**: 169–76.
19 Imai N, Ikeda K, Kawamura Y *et al.* Transcatheter arterial chemotherapy using miriplatin-lipiodol suspension with or without embolization for unresectable hepatocellular carcinoma. *Jpn J Clin Oncol* 2012; **42**: 175–82.
20 Imai N, Ikeda K, Seko Y *et al.* Previous chemoembolization response after transcatheter arterial chemoembolization (TACE) can predict the anti-tumor effect of subsequent TACE with miriplatin in patients with recurrent hepatocellular carcinoma. *Oncology* 2011; **80**: 188–94.
21 Imai Y, Chikayama T, Nakazawa M *et al.* Usefulness of miriplatin as an anticancer agent for transcatheter arterial chemoembolization in patients with unresectable hepatocellular carcinoma. *J Gastroenterol* 2011 [Epub ahead of print].
22 Lencioni R, Llovet JM. Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. *Semin Liver Dis* 2010; **30**: 52–60.
23 Marelli L, Stigliano R, Triantos C *et al.* Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. *Cardiovasc Intervent Radiol* 2007; **30**: 6–25.
24 Kamada K, Nakanishi T, Kitamoto M *et al.* Long-term prognosis of patients undergoing transcatheter arterial chemoembolization for unresectable hepatocellular carcinoma: comparison of cisplatin lipiodol suspension and doxorubicin hydrochloride emulsion. *J Vasc Interv Radiol* 2001; **12**: 847–54.
25 Kawamura Y, Ikeda K, Hirakawa M *et al.* Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin. *Hepatol Res* 2009; **39**: 346–54.
26 Imai N, Ikeda K, Seko Y *et al.* Transcatheter arterial chemotherapy with miriplatin for patients with hepatocellular carcinoma and chronic renal failure. *Nihon Shokakibyo Gakkai Zasshi* 2011; **108**: 1872–8.
27 Kishimoto S, Miyazawa K, Terakawa Y *et al.* Cytotoxicity of cis-[((1R,2R)-1,2-cyclohexanediamine-N,N')bis (myristato)]-platinum (II) suspended in Lipiodol in a newly established cisplatin-resistant rat hepatoma cell line. *Jpn J Cancer Res* 2000; **91**: 1326–32.
28 Miyayama S, Yamashiro M, Shibata Y *et al.* Comparison of local control effects of superselective transcatheter arterial chemoembolization using epirubicin plus mitomycin C and miriplatin for hepatocellular carcinoma. *Jpn J Radiol* 2012; **30**: 263–70.
29 Shim JH, Kim KM, Lee YJ *et al.* Complete necrosis after transarterial chemoembolization could predict prolonged survival in patients with recurrent intrahepatic hepatocellular carcinoma after curative resection. *Ann Surg Oncol* 2010; **17**: 869–77.

Original Paper

Intervirology

Intervirology 2012;55:417–425
DOI: 10.1159/000323526

Received: August 11, 2010
Accepted after revision: November 18, 2010
Published online: June 8, 2012

Amino Acid Substitution in HCV Core Region and Genetic Variation near the *IL28B* Gene Affect Viral Dynamics during Telaprevir, Peginterferon and Ribavirin Treatment

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Key Words
Hepatitis C virus · Core region · *IL28B* · Telaprevir · Peginterferon · Ribavirin · Viral dynamics

Abstract
Objectives: Genetic variation near the *IL28B* gene and substitution of aa 70 and 91 in the core region of HCV-1b are useful as predictors of treatment efficacy to telaprevir/pegylated interferon (PEG-IFN)/ribavirin, but its impact on viral dynamics is not clear. ***Methods:*** This study investigated predictive factors of viral dynamics during 12- or 24-week regimen of triple therapy in 80 Japanese adults infected with HCV-1b. ***Results:*** After 24 h of commencement of treatment, the proportion of patients with Arg70 and Leu91 substitutions in the core region who showed ≥3.0 log drop in HCV RNA level was significantly higher than that of patients with Gln70 (His70) and/or Met91. At 8 and 12 weeks, HCV RNA loss rate of patients with rs8099917 genotype TT near *IL28B* gene was significantly higher than that of patients with non-TT. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and SVR (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and SVR. ***Conclusions:*** This study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy.

Introduction

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1, 2]. At present, treatments based on interferon (IFN), in combination with ribavirin, are mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) in high viral loads (>100 kIU/ml) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis

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C [3]. Such a background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads [4, 5]. Recently, a new strategy was introduced in the treatment of chronic HCV infection by means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection [6]. Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity [7, 8]. Two previous studies (PROVE1 and PROVE2) showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin could achieve sustained virological response rates of 35–60 and 61–69% in patients infected with HCV-1, respectively [9, 10]. Furthermore, a recent study (PROVE3) also showed that the 24- and 48-week regimen of triple therapy could achieve sustained virological response rates of 51 and 53% in HCV-1 infected patients in whom initial PEG-IFN/ribavirin treatment failed, respectively [11].

Amino acid (aa) substitutions at positions 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy [12–14], and also affect clinical outcome, including hepatocarcinogenesis [15, 16]. Furthermore, genetic variations near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 as host-related factor, which encodes IFN-λ-3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1 [17–20], and also affect clinical outcome, including spontaneous clearance of HCV [21]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of sustained virological response to triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b [22]. However, it is not clear at this stage whether genetic variation near the *IL28B* gene and aa substitution of the core region can be used before therapy to predict viral dynamics during triple therapy.

The present study included 80 patients with HCV-1b and high viral loads, who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict viral dynamics during treatment, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near *IL28B* gene).

Patients and Methods

Study Population

Between May 2008 and September 2009, 81 patients infected with HCV were recruited to this study at the Department of Hepatology in Toranomon Hospital in metropolitan Tokyo. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave an informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN and ribavirin] (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

Eighty of the 81 patients met the following inclusion and exclusion criteria: (1) Diagnosis of chronic hepatitis C. (2) HCV-1b confirmed by sequence analysis. (3) HCV RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20–65 years. (6) Body weight ≥35 kg and ≤120 kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of ≤50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of ≥110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of ≥12 g/dl, neutrophil count ≥1,500/mm^3, and platelet count of ≥100,000/mm^3 at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. In this study, all of the 80 patients were evaluated for the pretreatment predictors for viral dynamics during triple therapy, and 77 of the 80 patients were followed up for at least 24 weeks after the completion of treatment. The treatment efficacy was evaluated by 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 or 500 mg three times a day at an 8-hour (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, N.J., USA) was injected subcutaneously at a median dose of 1.5 μg/kg (range 1.3–2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200–600 mg twice a day after breakfast and dinner (daily dose 600–1,000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil count or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1,500/mm^3, neutro-

Table 1. Profile and laboratory data at commencement of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b

Demographic data	
Number of patients	80
Sex, M/F	43/37
Age, years*	55 (23–65)
History of blood transfusion	24 (20.0%)
Family history of liver disease	13 (16.3%)
Body mass index*	22.5 (13.2–32.4)
*Laboratory data**	
Level of viremia, log IU/ml	6.8 (5.1–7.6)
Serum aspartate aminotransferase, IU/l	34 (15–118)
Serum alanine aminotransferase, IU/l	42 (12–175)
Serum albumin, g/dl	3.9 (3.3–4.6)
Gamma-glutamyl transpeptidase, IU/l	36 (9–229)
Leukocyte count, per mm^3	4,800 (2,800–8,100)
Hemoglobin, g/dl	14.3 (11.7–16.8)
Platelet count, $\times 10^4/mm^3$	17.3 (9.5–33.8)
α-Fetoprotein, μg/l	4 (2–39)
Total cholesterol, mg/dl	180 (112–276)
Fasting plasma glucose, mg/dl	92 (64–125)
Treatment	
PEG-IFNα-2b dose, μg/kg*	1.5 (1.3–2.0)
Ribavirin dose, mg/kg*	11.5 (7.2–18.4)
Telaprevir dose, 1,500/2,250 mg/day	10/70
Treatment regimen (T12PR12 group/T12PR24 group)	20/60
Amino acid substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	47/33
Core aa 91, leucine/methionine	43/37
ISDR of NS5A, wild-type/non-wild-type	76/4
Genetic variation near IL28B *gene*	
rs8099917 genotype, TT/TG/GG/ND	46/30/2/2
rs12979860 genotype, CC/CT/TT/ND	43/31/2/4
Past history of IFN therapy	
Treatment naive	27
Relapsers to previous treatment	33
Nonresponders to previous treatment	20

Data are numbers and percentages of patients, except those denoted by *, which represent the median (range) values.
ND = Not determined.

phil count below 750/mm^3 or platelet count below 80,000/mm^3; PEG-IFN was discontinued when these counts decreased below 1,000/mm^3, 500/mm^3 or 50,000/mm^3, respectively. When hemoglobin decreased to <10 g/dl, the daily dose of ribavirin was reduced from 600 to 400, 800 to 600 and 1,000 to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dl. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 80 patients at the commencement of treatment. They included 43 males and 37 females, aged 23–65 years (median 55 years).

Measurement of HCV RNA

The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment were evaluated at least once every month before, during, and after therapy. Furthermore, to investigate the pretreatment predictors for viral dynamics, HCV RNA levels during treatment were evaluated at 7 time points; 24 h, 1, 2, 4, 6, 8 and 12 weeks after the commencement of treatment. HCV RNA levels during treatment were evaluated in 80 (100%), 80 (100%), 80 (100%), 79 (98.8%), 75 (93.8%), 74 (92.5%), and 69 (86.3%) of the 80 patients, at the above time intervals, respectively. HCV RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as loss of HCV RNA. Especially, falls in HCV RNA levels at 24 h relative to baseline were investigated as very early dynamics.

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [23], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on 80 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) [12]. The sequence of 2209–2248 aa in the NS5A of HCV-1b (IFN sensitivity-determining region; ISDR) reported by Enomoto et al. [24] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (0, 1) or non-wild-type (≥2). In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing [22].

Genetic Variation near IL28B Gene

Samples for genomewide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of the Invader assay, TaqMan assay, or direct sequencing as described previously [25, 26].

In this study, genetic variations near *IL28B* gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome [17–22], were investigated.

Statistical Analysis

Nonparametric tests (χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to viral dynamics and sustained virological response. The ORs and 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 ($p < 0.05$) on univariate analysis were entered into

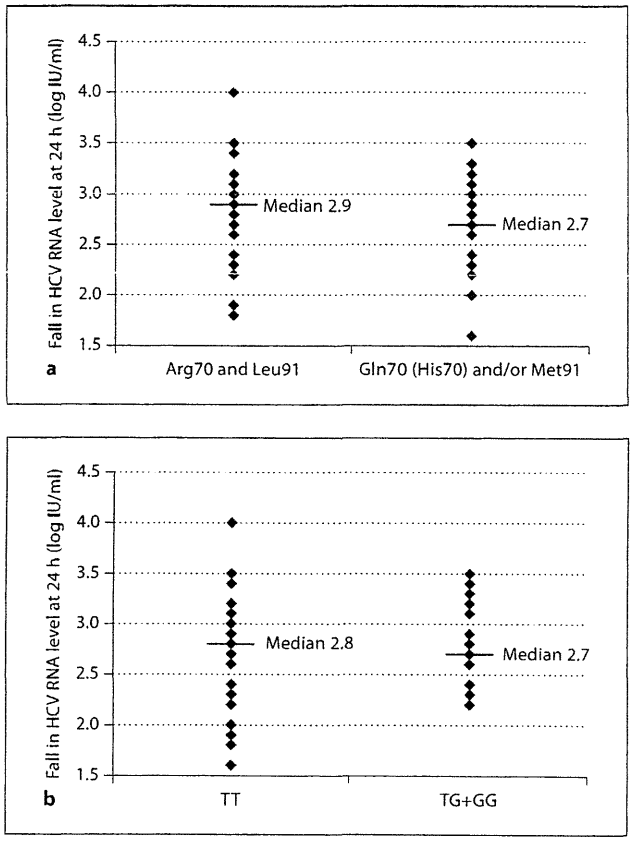

Fig. 1. a Very early dynamics according to amino acid substitutions in core region. After 24 h of commencement of the triple therapy, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml). **b** Very early dynamics according to genetic variation near the *IL28B* gene. After 24 h of commencement of the triple therapy, the decline of HCV RNA level of patients with rs8099917 genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml).

multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. The potential pretreatment factors associated with treatment efficacy included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase (γGTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, α-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the *IL28B* gene, and amino acid substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Virological Response to Therapy and Loss of HCV RNA during Treatment

Sustained virological response was achieved by 63.6% (49 of 77 patients). The disappearance rate of HCV RNA during treatment was 0% (0 of 80), 1.3% (1 of 80), 33.8% (27 of 80), 81.0% (64 of 79), 90.7% (68 of 75), 94.6% (70 of 74), and 89.9% (62 of 69) at 24 hours, 1, 2, 4, 6, 8, and 12 weeks, respectively.

Very Early Dynamics according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

After 24 h of commencement of the triple therapy, the proportion of patients with Arg70 and Leu91 substitutions who showed ≥3.0 log drop in HCV RNA level (45.2%; 14 of 31 patients) was significantly higher than that of patients with Gln70 (His70) and/or Met91 (14.3%; 7 of 49) ($p = 0.004$). Thus, patients with Arg70 and Leu91 (median 2.9 log IU/ml; range 1.8–4.0 log IU/ml) significantly showed the steeper decline of HCV RNA level than those with Gln70 (His70) and/or Met91 (median 2.7 log IU/ml; range 1.6–3.5 log IU/ml) (fig. 1a).

After 24 h of commencement of treatment, the proportion of patients with rs8099917 genotype TT who showed ≥3.0 log drop in HCV RNA level (30.4%; 14 of 46 patients) was not significantly different from that of patients with genotype TG and GG (21.9%; 7 of 32). Thus, the decline of HCV RNA level of patients with genotype TT (median 2.8 log IU/ml; range 1.6–4.0 log IU/ml) was not significantly different from that of patients with genotype TG and GG (median 2.7 log IU/ml; range 2.2–3.5 log IU/ml) (fig. 1b).

Hence, the fall in HCV RNA level at 24 h was influenced by aa substitution patterns in the core region, but was independent of genetic variation near *IL28B* gene.

Rates of Loss of HCV RNA according to Amino Acid Substitutions in Core Region and Genetic Variation near the IL28B Gene

According to the substitution of core aa 70 and 91, the rate of HCV RNA loss of patients with Arg70 and Leu91 was not significantly different from that of patients with

Gln70 (His70) and/or Met91 at each time point (1, 2, 4, 6, 8 and 12 weeks).

According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT (fig. 2).

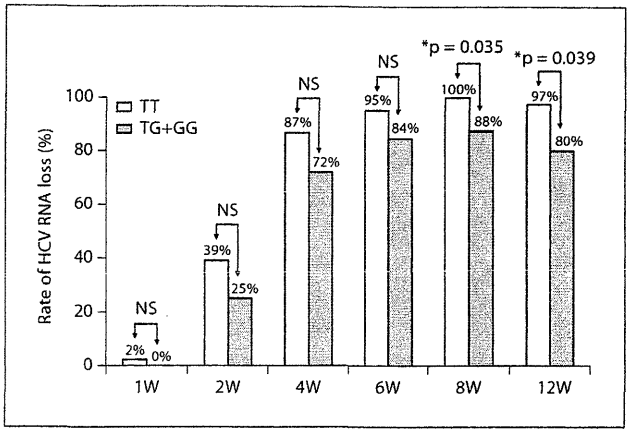

Fig. 2. Rates of loss of HCV RNA according to genetic variation near the *IL28B* gene. According to genetic variation near the *IL28B* gene, the rate of HCV RNA loss at 1, 2, 4 and 6 weeks was not significantly different between rs8099917 genotype TT and non-TT (TG and GG). However, at 8 and 12 weeks, the rate of HCV RNA loss of patients with genotype TT was significantly higher than that of patients with genotype non-TT.

Predictive Factors Associated with ≥3.0 log Fall in HCV RNA Level at 24 Hours

Univariate analysis identified two parameters that correlated with ≥3.0 log fall in HCV RNA level at 24 h significantly: substitution of aa 70 and 91 (Arg70 and Leu91; OR 4.94, p = 0.003) and body mass index (≥25.0; OR 3.92, p = 0.022). Two factors were identified by multivariate analysis as independent parameters that either significantly ($p < 0.05$) or marginally ($p < 0.10$) influenced ≥3.0 log fall in HCV RNA level at 24 h [Arg70 and Leu91 (OR 3.99, p = 0.015) and body mass index ≥25.0 (OR 3.24, p = 0.061)] (table 2).

Predictive Factors Associated with Loss of HCV RNA at 2, 4 and 12 Weeks

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 2 weeks significantly: platelet count (≥15.0 × 10^4/mm^3; OR 6.99, p = 0.014) and level of viremia (<7.0 log IU/ml; OR 3.13, p = 0.045). One factor was identified by multivariate analysis as independent parameter that either significantly or marginally influenced loss of HCV RNA at 2 weeks (platelet count ≥15.0 × 10^4/mm^3; OR 6.99, p = 0.014) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 4 weeks significantly: history of blood transfusion (absence; OR 5.71, p = 0.006) and body mass index (≥20.0; OR 4.29, p = 0.019). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 4 weeks (history of blood transfusion: absence; OR 4.29, p = 0.026, and body mass index ≥20.0; OR 3.47, p = 0.069) (table 2).

Univariate analysis identified two parameters that correlated with loss of HCV RNA at 12 weeks significantly: sex (male; OR 9.52, p = 0.043) and genetic variation in rs8099917 (genotype TT; OR 9.00, p = 0.048). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced loss of HCV RNA at 12 weeks (male sex; OR 11.0, p = 0.036, and rs8099917 genotype TT; OR 10.3, p = 0.042) (table 2).

Predictive Factors Associated with Sustained Virological Response

Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 3.51, p = 0.011), and genetic variation in rs8099917 (genotype TT; OR 11.1, $p < 0.001$) and rs12979860 (genotype CC; OR 10.2, $p < 0.001$). Two factors were identified by multivariate analysis as independent parameters that either significantly or marginally influenced sustained virological response (rs8099917 genotype TT; OR 9.94, $p<0.001$, and Arg70; OR 3.15, p = 0.055) (table 2).

Comparison of Factors Associated with Each Treatment Efficacy Identified by Multivariate Analysis

Table 3 shows independent parameters that either significantly or marginally influenced multivariate logistic regression for each evaluation of treatment efficacy. Multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 h (Arg70 and Leu91) and sustained virological response (Arg70), and rs8099917 (TT) as a predictor of HCV RNA loss at 12 weeks and sustained virological response. Thus, genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy.

Table 2. Factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b, identified by univariate and multivariate analysis

	Factor	Category	Univariate logistic regression		Multivariate logistic regression	
			OR (95% CI)	p	OR (95% CI)	p
A	≥3.0 log fall in HCV RNA at 24 h					
	Substitution of aa 70 and 91	1: Gln70 (His70) and/or Met91	1		1	
		2: Arg70 and Leu91	4.94 (1.70–14.4)	0.003	3.99 (1.31–12.2)	0.015
	Body mass index	1: <25.0	1		1	
		2: ≥25.0	3.92 (1.22–12.6)	0.022	3.24 (0.95–11.1)	0.061
B	HCV RNA loss at 2 weeks					
	Platelet count, $\times 10^4/mm^3$	1: <15.0	1		1	
		2: ≥15.0	6.99 (1.49–32.8)	0.014	6.99 (1.49–32.8)	0.014
	Level of viremia, log IU/ml	1: ≥7.0	1		–	–
		2: <7.0	3.13 (1.02–9.52)	0.045	–	–
C	HCV RNA loss at 4 weeks					
	History of blood transfusion	1: presence	1		1	
		2: absence	5.71 (1.66–19.6)	0.006	4.29 (1.86–15.6)	0.026
	Body mass index	1: <20.0	1		1	
		2: ≥20.0	4.29 (1.26–14.5)	0.019	3.47 (0.91–13.3)	0.069
D	HCV RNA loss at 12 weeks					
	Sex	1: female	1		1	
		2: male	9.52 (1.08–83.3)	0.043	11.0 (1.16–100)	0.036
	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	9.00 (1.02–79.5)	0.048	10.3 (1.08–98.0)	0.042
E	Sustained virological response					
	rs8099917 genotype	1: TG+GG	1		1	
		2: TT	11.1 (3.68–33.5)	<0.001	9.94 (3.05–32.4)	<0.001
	Substitution of aa 70	1: Gln70 (His70)	1		1	
		2: Arg70	3.51 (1.33–9.26)	0.011	3.15 (0.97–10.2)	0.055
	rs12979860 genotype	1: CT+TT	1		–	–
		2: CC	10.2 (3.33–3.13)	<0.001	–	–

Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors.

The other significant predictors of HCV RNA loss were platelet count ($\geq 15.0 \times 10^4/mm^3$) at 2 weeks, history of blood transfusion (absence) at 4 weeks, and sex (male) at 12 weeks.

Discussion

Thompson et al. [27] reported that genetic variation near *IL28B* gene was also associated with increased on-treatment and sustained virological response and effectively predicted treatment outcome in treatment-naive HCV-1 patients treated with PEG-IFN plus ribavirin. However, HCV RNA loss at 4 weeks (rapid virological response) was a strong predictor of sustained virological response regardless of genetic variation near the *IL28B* gene. This phenomenon probably explains why it might be important to identify the pretreatment factors that could predict viral dynamics during treatment. The present study is the first to identify the pretreatment factors that could predict viral dynamics during triple therapy in patients infected with HCV-1. These results should be interpreted with caution since races other than Japanese and the patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies including patients of other races and with HCV-1a to explore whether genetic variation near *IL28B* gene and aa substitution

Table 3. Comparison of factors associated with treatment efficacy of telaprevir, peginterferon and ribavirin triple therapy in Japanese patients infected with HCV-1b identified by multivariate analysis

Factor	≥3.0 log fall in HCV RNA (at 24 h)	HCV RNA loss (at 2 weeks)	HCV RNA loss (at 4 weeks)	HCV RNA loss (at 12 weeks)	Sustained virological response
Core aa 70 and 91	Arg70 and Leu91 p = 0.015 3.99 (1.31–12.2)*				Arg70 p = 0.055 3.15 (0.97–10.2)*
IL28B rs8099917				genotype TT p = 0.042 10.3 (1.08–98.0)*	genotype TT p < 0.001 9.94 (3.05–32.4)*
Others	body mass index p = 0.061 3.24 (0.95–11.1)*	platelet count p = 0.014 6.99 (1.49–32.8)*	body mass index p = 0.069 3.47 (0.91–13.3)* history of blood transfusion p = 0.026 4.29 (1.86–15.6)*	sex p = 0.036 11.0 (1.16–100)*	

Only variables that achieved statistical significance ($p < 0.05$) or marginal significance ($p < 0.10$) on multivariate logistic regression are shown. * OR (95% CI).

of core region also affect viral dynamics during triple therapy.

Two studies showed that aa substitution of the core region and genetic variation near *IL28B* gene affected viral dynamics during treatment, and sustained virological response to 48-week PEG-IFN plus ribavirin therapy in patients infected with HCV-1 [27, 28]. Furthermore, a recent report also showed that aa substitutions of core region might be used to predict very early dynamics (within 48 h) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin [29]. In the present study, multivariate analysis identified substitution of aa 70 and 91 as a predictor of ≥3.0 log fall in HCV RNA level at 24 hours (i.e. viral dynamics of very early phase) and sustained virological response, and rs8099917 as a predictor of HCV RNA loss at 12 weeks (i.e. viral dynamics of later phase) and sustained virological response. This study is the first to report that genetic variation near *IL28B* gene and aa substitution of the core region affect viral dynamics of different phases during triple therapy, and probably explains why the combination of these independent factors is very useful as pretreatment predictors of sustained virological response by triple therapy [22]. The underlying mechanisms of the different viral dynamics to treatment are still unclear, and further studies based on a larger number of patients are necessary to investigate the present results.

Previous data indicated that absence of advanced liver fibrosis and male gender were positive predictors of virological response to 48-week PEG-IFN plus ribavirin therapy [13, 28]. The present study also showed that higher levels of platelet count at 2 weeks, as a surrogate marker of milder liver fibrosis, and male gender at 12 weeks were significant positive predictors of HCV RNA loss during triple therapy. The other positive predictors were absence of history of blood transfusion at 4 weeks and higher levels of body mass index at 24 h and 4 weeks, but the underlying mechanisms are still unclear. Thus, this report identified the pretreatment factors that could predict viral dynamics during triple therapy, but this study, based on a small number of patients, might provide misleading results (e.g. possible type error). Further studies of a larger number of patients are required to explore predictors, including viral- and host-related factors.

The limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR) [30], were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation [31], were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both in vivo and in vitro studies [32, 33]. Thus, viral factors before and during triple therapy should be investigated in

future studies, and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, this study identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of viral dynamics during triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV-1b. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of the triple therapy, and further understanding of the complex interaction between virus- and host-related factors should facilitate the development of more effective therapeutic regimens.

Acknowledgment

This study was supported in part by a Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan.

References

1 Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D: Progress of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687–1695.

2 Kenny-Walsh E: Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin: Irish Hepatology Research Group. N Engl J Med 1999;340:1228–1233.

3 Tsubota A, Arase Y, Someya T, Suzuki Y, Suzuki F, Saitoh S, Ikeda K, Akuta N, Hosaka T, Kobayashi M, Kumada H: 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 2005;75:27–34.

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

5 Fried MW, Shiffman ML, Reddy R, Smith C, Marinos G, Gonçales FL, Häussinger 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.

6 Lin C, Kwong AD, Perni RB: Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. Infect Disord Drug Targets 2006;6:3–16.

7 Modi AA, Hoofnagle JH: New therapies for hepatitis C. Hepatology 2007;46:615–617.

8 Zeuzem S: Telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in chronic hepatitis C patients. J Hepatol 2008;49:157–159.

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

10 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: Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839–1850.

11 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: Telaprevir for previously treated chronic HCV infection. N Engl J Med 2010;362:1292–1303.

12 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: 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 2005;48:372–380.

13 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.

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

15 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 2007;46:1357–1364.

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

17 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: Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.

18 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;41:1105–1109.

19 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: IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104.

20 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;138:1338–1345.

21 Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M: Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801.

22 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: Amino acid substitution in HCV core region and genetic variation near *IL28B* gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 2010;52:421–429.
23 Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K: Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 1990;87:9524–9528.
24 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.
25 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;46:471–477.
26 Suzuki A, Yamada R, Chang X, et al: Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginie deminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;34:395–402.
27 Thompson AJ, Muir AJ, Sulkowski MS, et al: Interleukin-28B Polymorphism Improves Viral Kinetics and Is the Strongest Pretreatment Predictor of Sustained Virologic Response in Hepatitis C Virus-1 Patients. Gastroenterology 2010;139:120–129.
28 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686–1695.
29 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, Kumada H: 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: 575–582.
30 El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H: Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. Hepatology 2008;48:38–47.
31 Lin C, Gates CA, Rao BG, Brennan DL, Fulghum JR, Luong YP, Frantz JD, Lin K, Ma S, Wei YY, Perni RB, Kwong AD: In vitro studies of cross-resistance mutations against two hepatitis C virus serine protease inhibitors, VX-950 and BILN 2061. J Biol Chem 2005;280:36784–36791.
32 Forestier N, Reesink HW, Weegink CJ, McNair L, Kieffer TL, Chu HM, Purdy S, Jansen PL, Zeuzem S: Antiviral activity of telaprevir (VX-950) and peginterferon alfa-2a in patients with hepatitis C. Hepatology 2007;46: 640–648.
33 Zhou Y, Müh U, Hanzelka BL, Bartels DJ, Wei Y, Rao BG, Brennan DL, Tigges AM, Swenson L, Kwong AD, Lin C: Phenotypic and structural analyses of hepatitis C virus NS3 protease Arg155 variants: sensitivity to telaprevir (VX-950) and interferon alpha. J Biol Chem 2007;282:22619–22628.

Original Paper

Intervirology

Intervirology 2012;55:231–241
DOI: 10.1159/000328327

Received: September 22, 2010
Accepted after revision: March 28, 2011
Published online: July 5, 2011

Amino Acid Substitution in HCV Core/NS5A Region and Genetic Variation Near *IL28B* Gene Affect Treatment Efficacy to Interferon plus Ribavirin Combination Therapy

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Key Words

Hepatitis C virus · Interferon · Ribavirin · Core region · NS5A region · ISDR · IRRDR · *IL28B*

Abstract

Objective: To evaluate predictive factors of treatment efficacy to interferon (IFN)/ribavirin in patients infected with HCV genotype 1b (HCV-1b). ***Methods:*** This study investigated pretreatment predictors, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in 490 Japanese adults infected with HCV-1b. ***Results:*** The proportion of patients who showed end-of-treatment response (ETR), sustained virological response (SVR), and SVR after ETR was 76, 54, and 76%, respectively. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR. Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. Furthermore, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR. By multivariate analysis, rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core aa 70/91 was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. ***Conclusion:*** aa substitution in core/NS5A region and genetic variation near *IL28B* were important predictors of treatment efficacy to IFN/ribavirin.

Introduction

Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) combined with ribavirin carries potential serious side effects and is costly, especially when used long enough to achieve a high sustained virological response (SVR) in patients infected with HCV genotype 1b (HCV-1b) and high viral loads. For these rea-

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