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Determinants of Response to Triple Therapy of Telaprevir, Peginterferon, and Ribavirin in Previous Non-Responders Infected With HCV Genotype 1

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Patients who do not achieve sustained virological response to telaprevir/peginterferon (PEG-IFN)/ribavirin need to be identified. Predictive factors of virological response to the triple therapy in non-responders to previous PEG-IFN/ribavirin therapy are not clear. The aims of this study were to determine the predictive factors of virological response to a 24-week regimen of triple therapy in 15 non-responders to previous PEG-IFN/ribavirin therapy among 61 Japanese adults infected with HCV genotype 1. Overall, sustained virological response and end-of-treatment response were achieved by 27% and 60%, respectively. Telaprevir-resistant variants (by direct sequencing) appeared during or after treatment in 82% of patients who did not show sustained virological response, but disappeared at the end of study, except for one patient with resistant variant at baseline. Substitution at aa 70 (Arg70) and type of previous response to PEG-IFN/ribavirin (partial response) were identified as significant determinants of sustained virological response. In addition, alpha-fetoprotein level (<10 μg/L) and type of previous response (partial response) were identified as significant determinants of end-of-treatment response. Prediction of response to therapy based on the combination of these factors had high sensitivity, specificity, positive, and negative predictive values. In conclusion, this study identified amino acid substitution of the core region, alpha-fetoprotein level, and type of previous response as predictors of virological response to telaprevir/PEG-IFN/ribavirin in patients infected with HCV genotype 1b who had not responded to previous PEG-IFN/ribavirin therapy. ***J. Med. Virol. 84:1097–1105, 2012.*** © 2012 Wiley Periodicals, Inc.

KEY WORDS: HCV; core region; *IL28B*; telaprevir; peginterferon; ribavirin; partial response; null response; alpha-fetoprotein

INTRODUCTION

For chronic hepatitis C virus (HCV) infection, even when treated with the combination of peginterferon (PEG-IFN) and ribavirin, a sustained virological response lasting more than 24 weeks after withdrawal of treatment is achieved at most in 50% of patients with high viral load and infected with HCV genotype 1b (HCV-1b) [Manns et al., 2001; Fried et al., 2002]. Recently, new strategies were introduced for the treatment of chronic HCV infection based on inhibition of 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 [Lin et al., 2006]. Subsequent studies found that telaprevir, when combined with PEG-IFN and ribavirin, exhibited a robust antiviral activity [Modi and Hoofnagle, 2007; Zeuzem, 2008]. Two previous studies (PROVE1 and PROVE2) showed that the 12- and 24-week regimens of telaprevir/PEG-IFN/ribavirin achieved sustained virological response rates of 35–60% and 61–69%, respectively, in patients infected

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with HCV-1 [Hézode et al., 2009; McHutchison et al., 2009]. However, a recent study (PROVE3) also showed that the sustained virological response rates after 24- and 48-week regimens of the above triple therapy were lower (39% and 38%, respectively) in non-responders to previous PEG-IFN/ribavirin therapy infected with HCV-1, who did not achieve HCV-RNA negativity during or at the end of the initial combination therapy [McHutchison et al., 2010]. Furthermore, telaprevir-based regimen is reported to induce resistant variants [Lin et al., 2005; Kieffer et al., 2007], and side effects such as anemia and rash [Hézode et al., 2009; McHutchison et al., 2009, 2010]. Hence, prior non-responders, who do not achieve sustained virological response by triple therapy, need to be identified to avoid unnecessary side effects and appearance of telaprevir-resistant variants.

Amino acid (aa) substitutions at position 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 [Akuta et al., 2005, 2007a; Donlin et al., 2007], and also affect the clinical outcome, including hepatocarcinogenesis [Akuta et al., 2007b; Fishman et al., 2009]. Furthermore, *IL28B* genotype (rs8099917, rs12979860) on chromosome 19 as a host-related factor, which encodes IFN-λ-3, is a pretreatment predictor of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009], and also affect the clinical outcome, such as spontaneous clearance of HCV [Thomas et al., 2009]. Recent reports identified *IL28B* genotype and aa substitution of the core region as predictors of sustained virological response to telaprevir/PEG-IFN/ribavirin triple therapy in Japanese patients infected with HCV-1b [Akuta et al., 2010, 2011; Chayama et al., 2011]. However, it is not clear at this stage whether *IL28B* genotype and aa substitution of the core region can be used to predict the virological response to triple therapy in previous non-responders.

The aim of this study was to investigate the predictive factors of virological response to 24-week regimen of triple therapy in Japanese adult patients infected with HCV-1 who did not respond to previous dual PEG-IFN/ribavirin therapy.

PATIENTS AND METHODS

Study Patients

Between May 2008 and September 2009, 61 patients infected with HCV were recruited in this study at the Department of Hepatology, Toranomon Hospital, which is located 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 participation in this trial. Patients were assigned to a 24-week regimen of triple therapy [telaprevir (MP-424), PEG-IFN, and ribavirin] for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

Fifteen of 61 patients met the following inclusion and exclusion criteria: (i) diagnosis of chronic hepatitis C. (ii) HCV-1 confirmed by sequence analysis. (iii) HCV-RNA levels of $\geq$5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (iv) Japanese (Mongoloid) ethnicity. (v) Age at study entry of 20–65 years. (vi) Body weight $\geq$35 and $\leq$120 kg at the time of registration. (vii) Absence of decompensated liver cirrhosis. (viii) No detectable hepatitis B surface antigen (HBsAg) in serum. (ix) No history of hepato cellular carcinoma. (x) No previous treatment for malignancy. (xi) No history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (xii) No 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 $\leq$50 ml/min at baseline, diabetes requiring treatment or fasting glucose level of $\geq$110 mg/dl, autoimmune disease, cerebrovascular disorders, thyroid dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (xiii) Hemoglobin level of $\geq$12 g/dl, neutrophil count $\geq$1,500/mm^3, and platelet count of $\geq$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. (xiv) Previous non-responders, who did not achieve HCV-RNA negativity during or at the end of 24- to 48-week PEG-IFN plus ribavirin combination therapy. Previous non-response was defined as null response (a reduction of $<2\ \log_{10}$ in HCV-RNA during treatment) or partial response (a reduction of $2\ \log_{10}$ or more in HCV-RNA during treatment).

In this study, all of 15 patients were followed-up for at least 24 weeks after the completion of treatment. The treatment efficacy was evaluated by HCV-RNA negativity at the end of treatment (end-of-treatment response) and 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 mg three times a day at an 8-hr (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenklworth, NJ) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.3–1.7 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, neutrophil 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 mg, 800 to 600 mg 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 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 I. Profile and Laboratory Data at Commencement of Telaprevir, Peginterferon, and Ribavirin Triple Therapy of 15 Japanese Patients Infected With HCV Genotype 1, Who had been Non-Responders to Peginterferon Plus Ribavirin Combination Therapy

Demographic data	
n	15
Sex (M/F)	8/7
Age (years)*	56 (40–65)
History of blood transfusion	3 (20.0%)
Family history of liver disease	2 (13.3%)
Body mass index (kg/m^2)*	22.7 (18.1–26.5)
Laboratory data*	
HCV genotype (la/lb)	1/14
Level of viremia (log IU/ml)	6.6 (5.8–7.4)
Serum aspartate aminotransferase (IU/L)	36 (20–137)
Serum alanine aminotransferase (IU/L)	48 (17–136)
Serum albumin (g/dl)	3.9 (3.2–4.5)
Gamma-glutamyl transpeptidase (IU/L)	52 (20–154)
Leukocyte count (/mm^3)	4,700 (3,300–6,500)
Hemoglobin (g/dl)	14.4 (12.6–16.6)
Platelet count (×10^4/mm^3)	16.0 (9.1–23.9)
Alpha-fetoprotein (μg/L)	7 (2–38)
Total cholesterol (mg/dl)	178 (110–228)
Fasting plasma glucose (mg/dl)	89(81–111)
Treatment	
PEG-IFNα-2b dose (μg/kg)*	1.5 (1.3–1.7)
Ribavirin dose (mg/kg)*	11.8 (8.1–14.5)
Amino acid substitutions in the HCV genotype lb	
Core aa 70 (arginine/glutamine (histidine)/ND)	6/8/1
Core aa 91 (leucine/methionine/ND)	6/8/1
ISDR of NS5A (wild-type/non wild-type/ND)	13/1/1
IRRDR of NS5A (≤5/≥6/ND)	12/2/1
IL28B genotype	
rs8099917 genotype (TT/TG/GG)	1/12/2
rsl2979860 genotype (CC/CT/TT)	1/12/2
ITPA genotype	
rsl12735 genotype (CC/CA/AA)	14/1/0
Type of previous response to peginterferon/ribavirin	
Partial response/Null response	8/7

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

Table I summarizes the profiles and laboratory data of the 15 patients at the commencement of treatment. They included eight males and seven females, aged 40–65 years (median, 56 years). The present study was performed based on the Japanese patients infected with HCV-1b, except for one patient infected with HCV-1a.

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. 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 negative.

Assessments of Telaprevir-Resistant Variants

To analyze for resistant variants before, during, and after triple therapy, HCV-RNA was isolated from plasma, and the NS3/4A protease domains were amplified by reverse-transcriptase polymerase chain reaction assay and sequenced. Analyses were performed on baseline samples and in non-responders (HCV-RNA detectable during or at the end of treatment), viral breakthrough (re-elevation of viral loads before the end of treatment, even when HCV-RNA was temporarily negative during treatment), and relapse (re-elevation of viral loads after the end of treatment, even when HCV-RNA was negative at the end of treatment) by triple therapy. Telaprevir-resistant variants included V36A/M, T54A/S, R155I/K/M/T, and A156S/T/V [Kieffer et al., 2007]. In the present study, aa substitutions of NS3/4A were analyzed by direct sequencing.

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 [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 in a previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2209-2248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [1996] was determined, and the numbers of aa substitutions in ISDR were defined as wild-type (0, 1) or non wild-type (≥2) in comparison with HCV-J. Furthermore, the sequence of 2334-2379 aa in the NS5A of HCV-1b (IRRDR) reported by El-Shamy et al. [2008] was determined and then compared with the consensus sequence constructed in a previous study. In the present study, aa substitutions of the core region, and NS5A-ISDR/IRRDR of HCV-1b were analyzed by direct sequencing.

Determination of IL28B and ITPA Genotype

IL28B (rs8099917 and rs12979860) and *ITPA* (rs1127354) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003, 2011].

Statistical Analysis

Non-parametric tests (chi-squared test and Fisher's exact probability test) were used to determine those factors that significantly contributed to sustained virological response and end-of-treatment response. All P-values <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were determined. Each variable was transformed into categorical data consisting of two simple ordinal numbers for analyses. The potential pretreatment factors associated with sustained virological response and end-of-treatment response included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase, alanine aminotransferase, albumin, gamma-glutamyl transpeptidase, leukocyte count, hemoglobin, platelet count, HCV genotype, HCV-RNA level, alpha-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, type of previous response to PEG-IFN/ribavirin, *IL28B* and *ITPA* genotype, and amino acid substitution in the core region, and NS5A-ISDR/IRRDR. Statistical analyses were performed using the SPSS software (SPSS Inc., Chicago, IL). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated to determine the reliability of predictors of the response to therapy.

RESULTS

Virological Response to Therapy

Figure 1 shows the profile at commencement of triple therapy, virological course, and efficacy of treatment. The sustained virological response rates were 26.7% [four patients (Cases 1–4)], and the end-of-treatment response rates were 60.0% [nine patients (Case 1–9)]. Of the 11 patients (Cases 5–15) who did not show sustained virological response, the relapse, breakthrough, and non-response rates were 45.5% [five patients (Cases 5–9)], 36.4% [4 (Cases 10–13)], and 18.2% [2 (Cases 14, 15)], respectively. Three patients (Cases 10, 13, 15) stopped telaprevir before the completion of 12-week treatment (PEG-IFN and ribavirin continued), and one patient (9 weeks, Case 9) stopped the triple therapy before the completion of the 24-week regimen, due to a fall in Hb concentration.

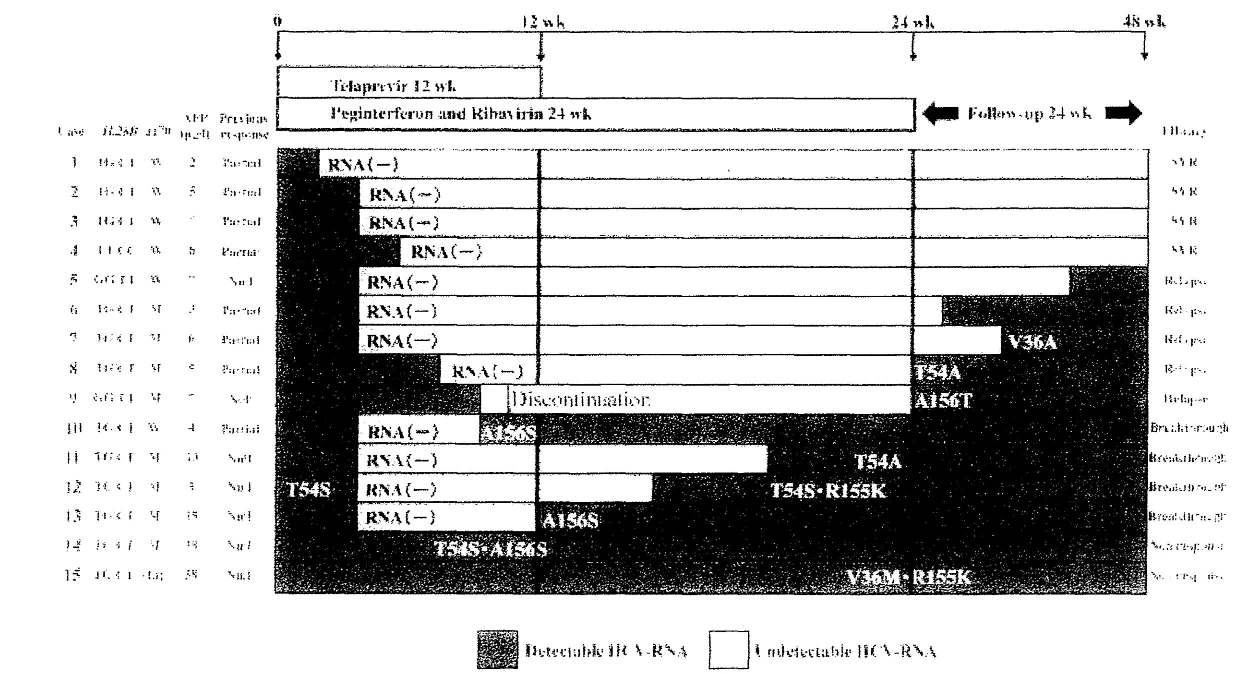

Fig. 1. Profiles at commencement of triple therapy, virological course, and treatment efficacy. The sustained virological response rates were 27%, and the end-of-treatment response rates were 60%. rs8099917/rs12979860 genotypes: *IL28B*, W: wild type (Arg70 substitution at core aa 70), M: mutant type (Gln70/His70). SVR: sustained virological response.

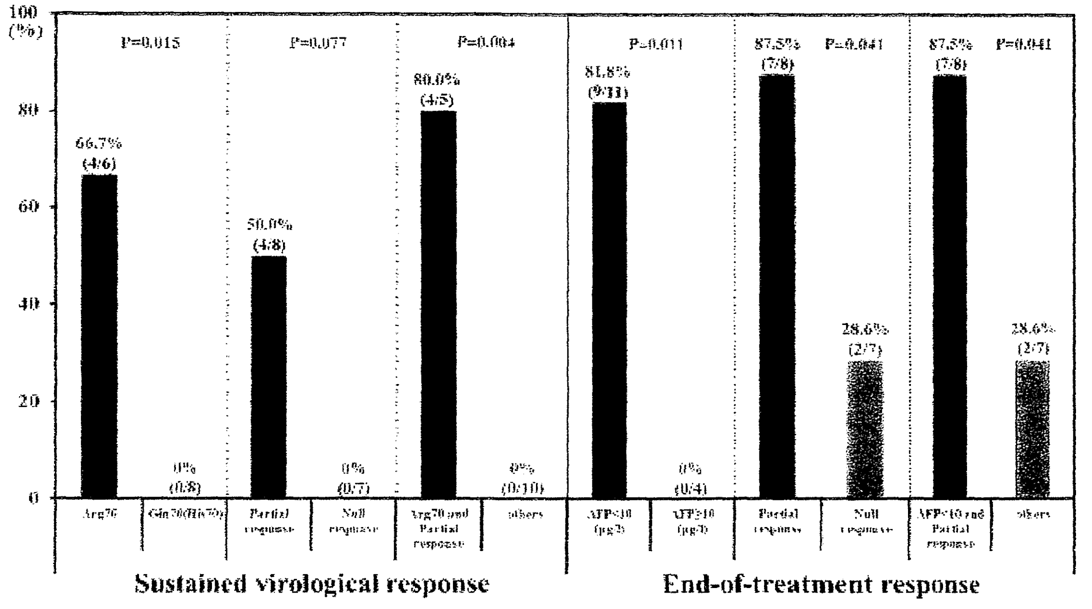

Fig. 2. Predictive factors associated with sustained virological response and end-of-treatment response to triple therapy. Arg70 and partial response are significant predictors of high-sustained virological response rate. Low level of alpha-fetoprotein and partial response are significant predictors of high end-of-treatment response rate.

Telaprevir-resistant variants were detected at baseline by direct sequencing in 6.7% [one patient (Case 12 with T54S)]. Of 11 patients who did not show a sustained virological response to triple therapy, telaprevir-resistant variants were detected during or after treatment in 81.8% [nine patients (Cases 7–15)], and not detected in 18.2% [two patients (Cases 5, 6)]. Resistant variants were consistent with those that have been reported previously [two patients with V36A/M (Cases 7, 15), four with T54A/S (Cases 8, 11, 12, 14), two with R155K (Cases 12, 15), and four with A156S/T (Cases 9, 10, 13, 14)] [Kieffer et al., 2007]. They were no longer detected by direct sequencing at 24 weeks after the completion of treatment, except for one patient with baseline-resistant variant (Case 12 with T54S).

Predictive Factors Associated With Sustained Virological Response

Fourteen of 15 patients showed *IL28B* rs8099917 non TT and rs12979860 non CC, whereas the other one patient (Case 4) had rs8099917 TT and rs12979860 CC. Thus, in non-responders to previous treatment, *IL28B* genotype did not play a role in sustained virological response.

The sustained virological response rate was significantly higher in patients with Arg70 [66.7% (four of six patients)] than in those with Gln70(His70) [0% (0 of 8)] ($P = 0.015$). Furthermore, the rate tended to be higher in patients with partial response to previous treatment [50.0% (four of eight patients)] than those with null response [0% (0 of 7)] ($P = 0.077$). Especially, the sustained virological response rate was significantly higher in patients with Arg70 plus partial response [80.0% (four of five patients)] than in other patients [0% (0 of 10)] ($P = 0.004$; Fig. 2). Thus, all four patients (100%) who achieved sustained virological response had Arg70 and showed partial response.

Predictive Factors Associated With End-of-Treatment Response

The end-of-treatment response rate was significantly higher in patients with low levels of alpha-fetoprotein [81.8% [9 of 11 patients)] than those with high levels of alpha-fetoprotein [0% (0 of 4)] ($P = 0.011$). Furthermore, the same rate was significantly higher in patients with partial response to previous treatment [87.5% (seven of eight patients)] than in those with null response [28.6% (two of seven patients)] ($P = 0.041$). The end-of-treatment response rate was also significantly higher in patients with low levels of alpha-fetoprotein plus partial response [87.5% (seven of eight patients)] than in others [28.6% (two of seven patients)] ($P = 0.041$; Fig. 2). Thus, seven of nine patients (77.8%) who achieved end-of-treatment response had low levels of alpha-fetoprotein and showed partial response. Inversely, all four patients (100%) with high levels of alpha-fetoprotein and null response did not achieve end-of-treatment response.

Assessment of Amino Acid Substitutions in Core Region and Type of Previous Response as Predictors of Sustained Virological Response

Next, the importance of substitution of core aa 70 and type of previous response to PEG-IFN/ribavirin in predicting sustained virological response were evaluated. The sustained virological response rate in patients with a combination of Arg70 or partial response was defined as PPV (prediction of sustained virological response), whereas the non-sustained virological response rate in patients with a combination of Gln70(His70) or null response was defined as NPV (prediction of non-sustained virological response).

In patients with Arg70, the sensitivity, specificity, PPV, and NPV for sustained virological response were 100%, 80.0%, 66.7%, and 100%, respectively. Therefore, Arg70 has high sensitivity, specificity, and NPV for prediction of sustained virological response. In patients with partial response, the sensitivity, specificity, PPV, and NPV were 100%, 63.6%, 50.0%, and 100%, respectively. Thus, partial response has high sensitivity and NPV in predicting sustained virological response. Furthermore, when both predictors were used, the sensitivity, specificity, PPV, and NPV were 100%, 90.9%, 80.0%, and 100%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of a sustained virological response (Table II).

Assessment of Alpha-fetoprotein and Type of Previous Response as Predictors of End-of-Treatment Response

The ability to predict end-of-treatment response by alpha-fetoprotein and type of previous response to PEG-IFN/ribavirin was evaluated. The end-of-treatment response rate in patients with a combination of low levels of alpha-fetoprotein (<10 μg/L) or partial response was defined as PPV (prediction of end-of-treatment response). The non end-of-treatment response rate of patients with a combination of high levels of alpha-fetoprotein (≥ 10 μg/L) or null response was defined as NPV (prediction of non end-of-treatment response).

In patients with low levels of alpha-fetoprotein, the sensitivity, specificity, PPV, and NPV for end-of-treatment response were 100%, 66.7%, 81.8%, and 100%, respectively. Thus, low level of alpha-fetoprotein has high sensitivity, PPV, and NPV for prediction of end-of-treatment response. In patients with partial response, the sensitivity, specificity, PPV, and NPV were 77.8%, 83.3%, 87.5%, and 71.4%, respectively. Thus, partial response has high sensitivity, specificity, and PPV in predicting end-of-treatment response. Furthermore, when both predictors were used, the sensitivity, specificity, PPV, and NPV were 80.0%, 100%, 100%, and 71.4%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of end-of-treatment response (Table III).

DISCUSSION

A recent study (PROVE3) reported low-sustained virological response rates (39% and 38%) for 24- and 48-week regimens of triple therapy, respectively, in previous non-responders infected with HCV-1 [McHutchison et al., 2010]. In the present study, the sustained virological response rate was also low (27%) in the T12PR24 group, similar to the above study. Four differences were evident between the present study and the above recent study: (i) the present study was based on a small number of non-responders. (ii) PEG-IFN was used in the above study at a fixed dose of PEG-IFNα-2a, whereas PEG-IFNα-2b was used at a body weight-adjusted dose in the present study. (iii) Body mass index of our patients (median; 23 kg/m^2) was lower than that of the participants of the recent study (median; >25 kg/m^2); and (iv) the present study included Japanese patients infected with HCV-1b, with the exception of one patient infected with HCV-1a. In another previous study (PROVE1), the viral breakthrough rate in HCV-1a subjects was higher than in HCV-1b, and this was due, at least in part, to the low genetic barrier to the emergence of the R155K variant in HCV-1a [Kieffer et al., 2007; McHutchison et al., 2009]. Further studies of larger number of patients matched for background, including genotype, race, and body mass index, as well as treatment regimen are required to determine the sustained virological response rate to triple therapy.

TABLE II. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Sustained Virological Response, According to Substitution of Core aa 70 and Type of Previous Response

	% (Number)			
	Sensitivity	Specificity	PPV	NPV
(A) Substitution at aa 70 of arginine (Arg70)	100 (4/4)	80.0 (8/10)	66.7 (4/6)	100 (8/8)
(B) Type of previous response (partial response)	100 (4/4)	63.6 (7/11)	50.0 (4/8)	100 (7/7)
(A) and (B)	100 (4/4)	90.9 (10/11)	80.0 (4/5)	100 (10/10)

PPV, sustained virological response rate for patients with a combination of Arg70 and partial response (prediction of sustained virological response). NPV, non-sustained virological response rates for patients with a combination of Gln70(His70) and null response (prediction of non-sustained virological response).

TABLE III. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for End-of-Treatment Response, According to Alpha-Fetoprotein and Type of Previous Response to Therapy

	% (Number)			
	Sensitivity	Specificity	PPV	NPV
(A) Alpha-fetoprotein (<10 μg/1)	100 (9/9)	66.7 (4/6)	81.8 (9/11)	100 (4/4)
(B) Type of previous response (partial response)	77.8 (7/9)	83.3 (5/6)	87.5 (7/8)	71.4 (5/7)
(A) and (B)	80.0 (8/10)	100 (5/5)	100 (8/8)	71.4 (5/7)

PPV, end-of-treatment response rates for patients with a combination of low levels of alpha-fetoprotein (<10 μg/L) and partial response (prediction of end-of-treatment response). NPV, non end-of-treatment response rates for patients with a combination of high levels of alpha-fetoprotein (≥10 μg/L) and null response (prediction of non end-of-treatment response).

The present study is the first to identify the pretreatment factors that can predict virological response to triple therapy in prior non-responders infected with HCV-1. The study identified substitution of aa70 (Arg70) and type of previous response (partial response) as predictors of sustained virological response in prior non-responders. The use of the combination of the above two predictors resulted in high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response. Especially, all four patients (100%) who achieved sustained virological response had the combination of Arg70 and partial response. Hence, the T12PR24 regimen might achieve a higher-sustained virological response rate in prior non-responders with the combination of Arg70 and partial response.

A recent study (REALIZE Study) showed that 59% of prior partial responders infected with HCV-1 achieved sustained virological response following 48-week regimen of triple therapy [Zeuzem et al., 2011]. In this regard, predictors of end-of-treatment response might be useful in selecting prior non-responders who could achieve sustained virological response following extension of the combination therapy to 48 weeks (T12PR48). The present study identified alpha-fetoprotein level (<10 μg/L) and type of previous response (partial response) as predictors of end-of-treatment response in previous non-responders. The combination of the above two predictors had high sensitivity, specificity, PPV, and NPV for prediction of end-of-treatment response. Especially, seven of nine patients (77.8%), who achieved end-of-treatment response were patients with low levels of alpha-fetoprotein and showed partial response. Hence, the T12PR48 regimen might achieve high-sustained virological response rates in prior non-responders who have low levels of alpha-fetoprotein and experienced partial response to prior therapy. All four patients (100%) who had high levels of alpha-fetoprotein and null response could not achieve end-of-treatment response. Thus, triple therapy might not achieve sustained virological response in prior non-responders with high levels of alpha-fetoprotein and history of null response, and the development of more effective therapeutic regimens is desirable for these patients in the future. This result should be interpreted with caution, since the present study was performed in Japanese patients infected with HCV-1b (with the exception of one patient infected with HCV-1a). Furthermore, the present study, based on a small number of patients, could not identify independent predictors by multivariate analysis. Any generalization of the results should await confirmation by a multicenter-randomized trial based on a larger number of prior non-responders, including patients of other races and those infected with HCV-1a.

The present study showed that high level of alpha-fetoprotein is a pretreatment predictor of poor virological response to triple therapy. Advanced liver fibrosis is usually associated with high levels of alpha-fetoprotein [Bayati et al., 1998; Chu et al., 2001; Hu et al., 2004]. Previous studies showed that high indocyanine green retention rates at 15 min (ICG R15) or low-serum albumin levels were also associated with advanced liver fibrosis, and that they were independent and significant predictors of poor virological response to PEG-IFN plus ribavirin combination therapy [Akuta et al., 2005, 2007a]. Further studies of large number of patients are required to explore the importance of various histopathological changes in the liver (including stage of fibrosis, platelet count, serum albumin, ICG R15, and alpha-fetoprotein), and to investigate the relationship between the severity of histopathological changes and the response to triple therapy.

The present study based on the direct sequencing identified the appearance of telaprevir-resistant variants during or after treatment in 82% of patients who did not show sustained virological response to triple therapy, but such variants were no longer detected at the end of the study except for one patient with baseline-resistant variant. The limitation of the present study was that the existence of minor clones of telaprevir-resistant variants could not be investigated. Further large-scale studies should be performed to investigate the effects of telaprevir-resistant variants on the response to treatment using the new drugs, including direct-acting antiviral therapy agents.

In conclusion, this study identified aa substitution of the core region, alpha-fetoprotein level, and type of previous response as predictors of virological response to treatment with telaprevir/PEG-IFN/ribavirin in previous non-responders infected with HCV-1b. Further large-scale prospective studies are necessary to confirm these findings, and to help in the design of more effective therapeutic regimens.

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Original Article

Prevalence and predictive factors of diabetes in hepatitis virus positive liver cirrhosis with fasting plasma glucose level of <126 mg/dL

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Aim: The aim of this study was to evaluate the prevalence and predictive factors of diabetes in hepatitis virus positive liver cirrhotic patients with fasting plasma glucose (FPG) level of <126 mg/dL.

Methods: A total of 263 patients with hepatitis C virus (HCV) or hepatitis B virus (HBV) positive liver cirrhosis, FPG level of <126 mg/dL, and had diabetes status evaluated by the use of 75-g oral glucose tolerance test (OGTT), were enrolled in this study. Plasma glucose and insulin levels were analyzed periodically for 3 h after oral glucose loading. Diabetes was defined as a 2-h post-load glucose on the OGTT of ≥200 mg/dL. The prevalence of diabetes by use of OGTT and predictive factors for diabetes were evaluated by the use of the Mann–Whitney *U*-test, Fisher's exact probability test or multivariate analysis by logistic regression. Hypoalbuminemia was defined as serum albumin level of <3.9 g/dL. Elevated indocyanine green retention rate at 15 min (ICG $_R$15) was regarded as ≥ 25%.

Results: Out of 263 patients, 44 (16.7%) were diagnosed as having diabetes. Multivariate analysis showed that diabetes occurred when patients had hypoalbuminemia of <3.9 g/dL (odds ratio [OR] 2.33; 95% confidential interval [CI] = 1.04–5.24; $P = 0.040$) and ICG $_R$15 of <25% (OR 2.36; 95%CI = 1.01–5.58).

Conclusions: Hypoalbuminemia and elevated ICG $_R$15 in hepatitis virus related cirrhotic patients with FPG level of <126 mg/day enhance diabetes pattern after OGTT with significant difference.

Key words: diabetes mellitus, hepatitis virus, liver cirrhosis, oral glucose tolerance test

INTRODUCTION

HEPATITIS C VIRUS (HCV) is one of the more common causes of chronic liver disease worldwide. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis and/or hepatocellular carcinoma over a period of 10–30 years.[1–8] Lately, it have been reported that chronic HCV infection is associated with type 2 diabetes mellitus (T2DM).[9–11] Moreover, T2DM has been suggested to enhance with the development of HCC and poor prognosis of liver transplantation.[12–15] Thus, early intervention to prevent or improve T2DM is necessary to get good prognosis in HCV patients.

However, the big problem in chronic liver disease is that fasting serum glucose (FPG) often shows normal level. Hence, examination of oral glucose tolerance test (OGTT) is necessary to evaluate diagnosis of precise diabetes in patients with chronic liver disease. With this background, we evaluated the prevalence of abnormal glucose state and predictive factors for diabetes in HCV positive liver cirrhosis patients with fasting plasma glucose level of <126 mg/dL. We investigated the

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prevalence of abnormal glucose state and predictive factors for diabetes in hepatitis B virus (HBV) positive patients with liver cirrhosis and compared with HCV.

METHODS

Patients

A TOTAL OF 263 Japanese patients who were diagnosed with liver cirrhosis by laparoscopic and/or histological findings from December 1998 to January 2005 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan were enrolled. Inclusion criteria were as follows: (i) evidence of liver cirrhosis by laparoscopy and/or histological findings; (ii) FPG level of <126 mg/dL; (iii) evidence of HCV or HBV by serum examination; (iv) negativity for antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by indirect immunofluorescence assay; (v) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; (vi) no underlying systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis. Patients with any of the following criteria were excluded from the study: (i) advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites, (ii) a history of diabetes, (iii) taking medicines that may influence on glucose tolerance such as branched chain amino acid (BCAA), thiazide diuretics, and angiotensin receptor antagonist.

A total of 263 patients with FPG of <126 mg/dL undertook a 75-g OGTT. Plasma glucose levels were analyzed periodically for 3 h after oral glucose loading. Impaired glucose tolerance (IGT) were defined as a 2-h post-load glucose on the OGTT of ≥140 mg/dL, but <200 mg/dL. Diabetes was defined as a 2-h post-load glucose on the OGTT of ≥200 mg/dL. T2DM was diagnosed by the use of the 2003 criteria of the American Diabetes Association.[16]

The index of insulin resistance was calculated on the fasting glucose and insulin by the homeostasis model for insulin resistance (HOMA-IR). Insulin secretion was calculated by the insulinogenic index (IGI); IGI = (Ins30-Ins0)/(Glc30-Glc0), Ins0: fasting plasma insulin (mU/L); Ins_{30}: insulin 30 min after glucose intake (IU/mL); Glc0: fasting plasma glucose (mg/dL); and Glc30: plasma glucose 30 min after glucose intake (mg/dL).

The physicians in charge explained the purpose and method of the OGTT to each patient. Informed consent was obtained from all patients included in the present study. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study had been approved by the Institutional Review Board of our hospital.

Clinical and laboratory analysis

Anthropometric analysis included height, weight and body mass index (BMI), and the latter was calculated as weight (kg) divided by the square of the height (m^2). Laboratory analysis was performed via standard laboratory methods. The biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total cholesterol, high density lipoprotein (HDL) cholesterol, triglyceride, albumin, platelet, fasting plasma glucose (FPG) and fasting insulin. Serum insulin levels were measured with a solid-phase radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Hypoalbuminemia was defined as serum albumin level of <3.9 g/dL. Elevated indocyanine green retention rate at 15 min (ICG $_R$15) was regarded as ≥25%.

Laboratory investigation

Hepatitis B surface antigen (HBsAg) was assayed using commercially available radioimmunoassay kits. Antibody against HCV was detected with a third-generation enzyme-linked immunoassay. HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche, Tokyo, Japan). HbA1c was measured using a high performance liquid chromatography (HPLC) method. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg)/height (in m^2).

Statistical analysis

The results are presented as means ± standard deviation (SD) or as numbers. Statistical differences in quantitative data were determined using the Mann–Whitney *U*-test, Fisher's exact probability test or multivariate analysis by logistic regression.

Multivariate analysis for diabetes was carried out by logistic regression. The Statistical Program for Social Sciences software package (SPSS 11.5 for Windows, SPSS, Chicago, IL, USA) was used to perform statistical analysis. A *P*-value < 0.05 was considered to be statistically significant.

RESULTS

Patients' characteristics

TABLE 1 SHOWS the characteristics at the day of evaluating OGTT in the 263 enrolled patients. The

Table 1 Clinical characteristics of cirrhotic patients with hepatitis C virus (HCV)†

Characteristic	
n	263
Sex (male/female)	178/85
Age (years)	51.6 ± 11.2
Body mass index	21.8 ± 3.0
HBV/HCV	96/167
Fasting plasma glucose (mg/dL)	84 ± 13
Albumin (g/dL)	4.1 ± 0.5
Total cholesterol (g/dL)	163 ± 37
HDL cholesterol (g/dL)	47 ± 13
Triglyceride (mg/dL)	98 ± 35
Uric acid (mg/dL)	5.2 ± 1.2
AST (IU/L)	78 ± 70
ALT (IU/L)	72 ± 68
GGT (IU/L)	74 ± 50
Platelet (×104/mm^3)	11.3 ± 4.1
ICG$_R$15 (%)	25.4 ± 14.0

†Data are number of patients or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; HBV, hepatitis B virus; HDL, high density lipoprotein; ICG$_R$15; indocyanine green retention rate at 15 min.

mean age was 51.6 years and mean FPG level was 84 mg/dL. The serum albumin level was 4.1 ± 0.5 g/dL and ICG$_R$15 was 26.5 ± 14.0%. On the diagnosis of liver cirrhosis, 160 of 263 patients were diagnosed by laparoscopy and liver biopsy; 69 patients were diagnosed by laparoscopy only; 34 patients were diagnosed by biopsy only.

Prevalence of IGT and diabetes in hepatitis virus positive liver cirrhosis with FPG of <126 mg/dL

Out of 263 patients who had hepatitis virus-related liver cirrhosis with FPG of <126 mg/dL, 44 (16.7%) patients were diagnosed as having DM and 73 (27.8%) patients were diagnosed as having IGT. Table 2 shows the predictive factors for DM pattern by the use of OGTT in hepatitis virus related cirrhotic patients. Multivariate analysis showed that diabetes occurred when patients had hypoalbuminemia of <3.9 g/dL (odds ratio [OR] 2.33; 95% confidential interval [CI] = 1.04–5.24; $P = 0.040$) and ICG $_R$15 of <25% (OR 2.36; 95%CI = 1.01–5.58). Table 3 shows the incidence of diabetes based on serum albumin and ICG $_R$15. The incidence of diabetes in patients with hepatitis virus related liver cirrhosis was 5.8% (7/120) in group A with serum albumin level of ≥3.9 g/dL and ICG $_R$15 of <25%. On the other hand, that was 35.6% (21/59) in group B with hypoalbuminemia of <3.9 g/dL and ICG $_R$15 of ≥25%.

Changes of glucose state based on difference of serum albumin level

Table 4 shows the glucose and insulin dynamics after OGTT in cirrhotic patients that belonged to group A with serum albumin level of ≥3.9 g/dL and ICG $_R$15 of

Table 2 Predictive factors for diabetes in cirrhotic patients with hepatitis C virus (HCV)

Variables	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age (per 10 years)	1.50 (1.04–2.16)	0.031		
Gender (M/F)	1.08 (0.52–2.23)	0.842		
Body mass index (per 5)	1.18 (0.59–2.36)	0.631		
HCV/HBV	2.38 (1.05–5.43)	0.039		
AST (IU/L, ≥37/<37)	1.01 (0.45–2.25)	0.996		
ALT (IU/L, ≥42/<42)	0.81 (0.40–1.65)	0.563		
GGT (IU/L, ≥109/<109)	1.89 (0.66–5.45)	0.238		
Platelet (×10^4/mm^3, <10/≥10)	2.59 (1.26–5.32)	0.009		
Albumin (g/dL, <3.9/≥3.9)	3.40 (1.66–6.94)	0.001	2.33 (1.04–5.24)	0.040
Triglyceride (mg/dL, ≥150/<150)	2.26 (0.74–6.90)	0.152		
Total cholesterol (mg/dL, ≥180/<180)	0.69 (0.30–1.60)	0.387		
HDL cholesterol (mg/dL, <40/≥40)	1.09 (0.43–2.73)	0.857		
ICGR15 (%, ≥25/<25)	3.64 (1.67–7.95)	0.001	2.36 (1.01–5.58)	0.049

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; HBV, hepatitis B virus; HDL, high density lipoprotein; ICG$_R$15, indocyanine green retention rate at 15 min; OR, odds ratio.

Table 3 Diabetic rate based on serum albumin and ICG $_{R}$15

	Albumin; ≥3.9 g/dL	Albumin; <3.9 g/dL	Total
ICG $_{R}$15 < 25 (%)	5.8% (7/120)	15.0% (3/20)	7.1% (10/140)
ICG $_{R}$15 ≥ 25 (%)	20.3% (13/64)	35.6% (21/59)	27.6% (34/123)
Total	10.9% (20/184)	30.4% (24/79)	16.7% (44/263)

ICG$_{R}$15, indocyanine green retention rate at 15 min.

<25% or group B with serum albumin level of <3.9 g/dL and ICG $_{R}$15 of ≥25%. The serum glucose levels at 0, 60, 90, 120, and 180 min after the initiation of OGTT in patients with serum albumin level of <3.9 g/dL and ICG $_{R}$15 of ≥25% were statistically higher than those in patients with serum albumin level of ≥3.9 g/dL and ICG $_{R}$15 of <25%. HOMA-IR in patients with serum albumin level of <3.9 g/dL and ICG $_{R}$15 of ≥25% was higher than that in patients with serum albumin level of ≥3.9 g/dL and ICG $_{R}$15 of <25%. IGI in patients with serum albumin level of <3.9 g/dL and ICG $_{R}$15 of ≥25% was lower than that in patients with serum albumin level of ≥3.9 g/dL and ICG $_{R}$15 of <25%.

DISCUSSION

WE HAVE DESCRIBED the prevalence of abnormal glucose state and predictive factors for diabetes in HCV or HBV positive liver cirrhosis patients with fasting plasma glucose level of <126 mg/dL in the present study. Enrolled patients had liver cirrhosis diagnosed with laparoscopy and/or histological examination. There are sometimes discrepancies between laparoscopic finding and histological findings in patients with HCV.[17] Thus, in the present study, cirrhotic patients diagnosed by either laparoscopy and/or histological examination were enrolled.

The present study shows several findings with regard to the prevalence of abnormal glucose state in hepatitis virus related cirrhotic patients with FPG level of <126 mg/dL. First, approximately 17% of the cirrhotic patients with FPG level of <126 mg/dL had diabetic pattern by the OGTT. If OGTT was not performed in patients who were diagnosed as having diabetes after OGTT, diabetes would be missed.

Second, multivariate analysis suggested that lower serum albumin level and elevated ICG$_{R}$15 were independent risk factors of diabetes mellitus. Our result shows that patients with hypoalbuminemia and elevated ICG$_{R}$15 should pay attention to complication of T2DM even if FPG is in the normal range. In the present study, hypoalbuminemia was defined as serum albumin level of <3.9 g/dL and elevated ICG $_{R}$15 was regarded as ≥25%. As the serum albumin level (mean ± standard deviation)

Table 4 Glucose and Insulin dynamics after oral glucose tolerance test (OGTT) in cirrhotic patients

	Group A (albumin; <3.9 g/dL) (ICG $_{R}$15; ≥25%)	Group B (albumin; ≥3.9 g/dL) (ICG $_{R}$15; <25%)	*P*-value
Number	59	120	
HOMA-IR	3.22 ± 2.24	2.14 ± 1.12	0.003
IGI	0.70 ± 0.53	0.96 ± 0.83	0.042
Glucose (mg/dL)			
At 0 min	89.3 ± 12.0	82.8 ± 10.3	0.034
At 30 min	170.3 ± 42.1	159.4 ± 31.4	0.058
At 60 min	200.6 ± 70.7	168.0 ± 49.9	0.020
At 90 min	206.3 ± 64.8	168.0 ± 62.0	0.002
At 120 min	179.7 ± 68.5	136.3 ± 49.0	<0.001
At 180 min	153.4 ± 64.2	117.7 ± 59.3	<0.001
Insulin (mI/L)			
At 0 min	14.1 ± 9.4	10.4 ± 5.1	0.011
At 30 min	72.1 ± 36.3	75.1 ± 31.9	0.758
At 120 min	138.4 ± 76.5	102.1 ± 62.8	0.004

HOMA-IR, homeostasis model for insulin resistance; IGI, insulinogenic index.

of the approximately 70 000 subjects without liver damage and kidney damage in our hospital was 4.5 ± 0.3 g/dL, lower limit of normal albumin level was defined as 3.9 g/dL (=mean−2 × standard deviation). On ICG_R15, we divided the patients into two groups based on mean level of 25%. The hypoalbuminemia and elevated ICG_R15 indicates the severity of liver cirrhosis. Thus, our results suggest that severity of liver cirrhosis was the most important factor for predicting T2DM. The reported predictive factors of diabetes mellitus in liver cirrhosis were age, male, BMI, and Child–Pugh score.[9,10,18–21] Quintana *et al.* have reported hypoalbuminemia as risk factor of diabetes mellitus in cirrhotic patients.[22] On the other hand, EL-Serag *et al.* have reported that hepatogeneous diabetes is less frequently associated with risk factors such as age, BMI, and family history of diabetes.[23]

Third, patients with serum albumin level of <3.9 g/dL and ICG_R15 of ≥25% revealed high insulin resistance and low insulin secretion compared to patients with serum albumin level of ≥3.9 g/dL and ICG_R15 of <25%. This result suggests that insulin resistance and insulin secretion are associated with the onset of diabetes in advanced liver cirrhosis.

The precise mechanism of hepatogeneous diabetes is not precisely known. The possible mechanism is the following: (i) insulin resistance of muscle and adipose tissue; and (ii) impairment of the insulin secretion activity of the beta-cells of the pancreas.[24,25] Our results show the elevated insulin resistance and decrease of insulinogenic index. Thus, our results agreed with the possible mechanism of hepatogeneous diabetes.

The limitation of present study is that our cohort contains Japanese patients only. Thus, the result needs to be confirmed in other ethnic groups. Moreover, in patients with chronic liver disease, HbA1c levels have been seen to be apparently lower than real values due to a shortened half-life of erythrocytes originating from hypersplenism.[26] Thus, we could not evaluate the HbA1c in the present study.

In conclusion, our data suggest that physicians in charge of hepatitis virus related cirrhotic patients with hypoalbuminemia and elevated ICG_R15 should pay attention to complication of diabetes.

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Transcatheter Arterial Chemotherapy Using Miriplatin–Lipiodol Suspension with or without Embolization for Unresectable Hepatocellular Carcinoma

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Objective: The purpose of this retrospective study was to compare the anti-tumor and adverse effects of transcatheter arterial chemoembolization and transcatheter arterial infusion chemotherapy using miriplatin–lipiodol suspension in patients with unresectable hepatocellular carcinoma.

Methods: From 2007 to 2010, 162 consecutive patients with unresectable hepatocellular carcinoma were treated using miriplatin. Of these, 122 patients were treated by transcatheter arterial chemoembolization and 40 were treated by transcatheter arterial infusion chemotherapy. There were no significant differences in baseline characteristics between the two groups, except for prothrombin activity. Assessments were performed 1–3 months after treatment.

Results: Objective responses were achieved in 13 patients undergoing transcatheter arterial infusion chemotherapy and 70 patients undergoing transcatheter arterial chemoembolization (33 versus 57%, $P = 0.003$). By multivariate logistic regression analysis, objective response was significantly associated with (i) a *Lens culinaris* agglutinin-reactive fraction of α-fetoprotein $\leq$10% ($P = 0.004$; risk ratio = 3.09; 95% confidence interval = 1.42–6.70), (ii) no previous transcatheter arterial chemoembolization ($P = 0.007$; risk ratio = 4.41; 95% confidence interval = 1.49–13.07) and (iii) transcatheter arterial chemoembolization using gelatin sponge 1 mm particles ($P = 0.021$; risk ratio = 2.97; 95% confidence interval = 1.17–7.49). Fever, anorexia and elevated serum transaminase levels were observed in most patients after miriplatin administration; there were no significant differences in the number of adverse effects between the two groups.

Conclusions: These results suggest that the addition of embolizing agents to a treatment regimen using miriplatin–lipiodol suspension can be safely used for patients with unresectable hepatocellular carcinoma. Objective response was achieved in a significantly higher number of transcatheter arterial chemoembolization patients than transcatheter arterial infusion chemotherapy patients.

Key words: miriplatin – hepatocellular carcinoma – transcatheter arterial chemoembolization

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide (1). Since it is well known that more than 80% of HCC cases are associated with liver cirrhosis, routine clinical evaluations of cirrhotic patients that include ultrasound could potentially lead to the detection of small HCCs (2–4). Curative therapies, including resection, liver transplantation and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation), are applicable to only 30–40% of HCC patients. For patients with advanced HCC, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option (5–12).

Although many chemotherapeutic agents, including doxorubicin, epirubicin, mitomycin C and cisplatin, are used with lipiodol, a lipid lymphographic agent consisting of ethyl esters of iodized fatty acids from poppy seed oil (Lipiodol Ultra-Fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France), the best choices for first- and second-line drugs for TACE remain uncertain (13–15). Miriplatin (*cis*-[((1*R*,2*R*)-1,2-cyclohexanediamine-*N*,*N'*)bis(myristato)]-platinum(II)monohydrate; Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol (16–19). When lipiodol is injected into an artery supplying HCC nodules, it selectively accumulates in the tumor. A miriplatin–lipiodol suspension deposited within HCC nodules will gradually release active platinum compounds into tumor tissues. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC, but the addition of embolizing agents to miriplatin–lipiodol suspension has not been evaluated (20,21). We hypothesized that the addition of embolizing agents to miriplatin–lipiodol suspension would increase the anti-tumor effects in patients with HCC. The purpose of this retrospective study was to compare the anti-tumor effects and adverse effects of TACE and transcatheter arterial infusion chemotherapy (TAI) using miriplatin–lipiodol suspension in patients with unresectable HCC.

PATIENTS AND METHODS

Study Population

From December 2007 to December 2010, 162 consecutive patients with unresectable HCC were treated using transcatheter arterial chemotherapy with a miriplatin–lipiodol suspension at our institution. Of these, 122 patients were treated using TACE and 40 were treated using TAI. The patients were divided into two groups primarily based on when they were treated. After the approval of miriplatin in Japan, miriplatin was initially administered by TAI. After our experience using TAI plus miriplatin, TACE was used with miriplatin. Patients in the TAI group were mainly treated from January 2010 to May 2010, and patients in the TACE group were mainly treated from June 2010 to December 2010. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

Characterization of Hepatocellular Carcinomas

Before treatment with miriplatin, all patients underwent comprehensive evaluations consisting of medical history, physical examination, measurement of tumor size, assessment of performance status, chest radiography, liver imaging studies [dynamic computerized tomography (dynamic CT), ultrasonography (US), digital subtraction angiography (DSA)], complete blood count and blood chemistries.

The clinical diagnosis of HCC was based on the findings of dynamic CT, US or DSA and increased serum levels of α-fetoprotein (AFP) and/or des-γ-carboxyprothrombin (DCP). Imaging studies included triphasic contrast-enhanced CT with bolus contrast injection and CT during arterial portography combined with CT during hepatic arteriography at the time of TACE. Lesions that appeared hypervascular during the arterial phase and that had relatively low density on the portal venous phase were diagnosed as HCC. Patients who had extrahepatic metastases of HCC or other malignancies were excluded.

Tumor staging was performed according to the criteria of the Liver Cancer Study Group of Japan (22) and was based on the following three criteria: (i) solitary tumor, (ii) <2 cm in diameter and (iii) no vessel invasion. Stage I (T1) was defined as fulfilling all three criteria; Stage II (T2) as fulfilling two criteria; Stage III (T3) as one of three criteria; and Stage IVA (T4) as none of the three criteria with no distant metastasis, or any T factor with lymph node metastasis; and Stage IVB as any T factor with distant metastasis.

There were 129 patients (80%) who had undergone previous TACE. Among these patients, the median number of TACE procedures was 4 (range, 1–13), and the median interval between the last previous TACE procedure and miriplatin administration was 4 months (range, 1–41).

Treatments

Patients were hydrated through a peripheral line. The femoral artery was catheterized after administering local anesthesia, and the catheter was inserted super-selectively into the hepatic artery that supplied the target tumor. The dosage of miriplatin was limited to 120 mg. The miriplatin–lipiodol suspension was slowly administered under fluoroscopic guidance. In the TAI patients, the miriplatin–lipiodol suspension was administered through tumor-supplying vessels until stasis and reflux were achieved. In the TACE patients, 1 mm gelatin sponge particles (Gelpart; Nippon Kayaku, Tokyo, Japan) were injected after the administration of the miriplatin–lipiodol suspension until stasis and reflux were achieved. TACE was performed for patients without thrombus of the main portal vein and severe liver dysfunction. Each dose of miriplatin–lipiodol was determined according to the size of the tumor and the degree of liver dysfunction.

Assessment of Therapeutic Effect

The effect of chemotherapy was evaluated by dynamic CT 1–3 months after TACE or TAI and was based on changes in the maximum diameters of the viable target lesions, that is, lesions showing enhancement in the arterial phase. The categories of responses were based on the modified Response Evaluation Criteria in Solid Tumors (mRECIST) 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 (enhancement in the arterial phase) target lesions; stable disease (SD) = any patient not qualifying for either PR or progressive disease (PD); and PD = an increase of at least 20% in the sum of the diameters of viable target lesions (23).

Toxicity Evaluations

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0. Within 2 weeks before TACE or TAI with miriplatin, and at 3–7 days and 1 month after the procedures, the following toxicity evaluations were made: hematological assessments (leukocyte and thrombocyte counts) and clinical chemistry assessments [serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), albumin, total bilirubin and prothrombin activity (PT)]. The indocyanine green retention rate at 15 min (ICG-R15) was assessed before and at 1 week after miriplatin administration.

Statistical Analysis

Distributions of patient characteristics were assessed by the χ^2 test, the Mann–Whitney U-test, the Friedman test, the Wilcoxon signed-rank test and Bonferroni adjustments as appropriate. Multivariate logistic regression analysis was used to determine significant predictors for objective response (CR or PR). All variables were expressed as medians (minimum–maximum). All tests were two-sided, and P values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., IBM; Somers, New York, USA).

RESULTS

Patient Characteristics

The clinical characteristics of the treatment groups are summarized in Table 1. At the time of miriplatin administration, there were 117 (72%) Child–Pugh class A patients and 45 (28%) class B patients. There were no significant differences between the two groups for any baseline characteristics except for PT.

There were 27 patients diagnosed with a solitary tumor, and 135 patients diagnosed with multiple tumors. The median maximum tumor size was 20 mm (range, 7–100 mm). There were 15 patients with Stage I, 75 patients with Stage II, 64 patients with Stage III and 8 patients with Stage IVA tumors.

One hundred and twenty-nine of 162 patients (80%) had a history of TACE, 108 (67%) patients previously received TACE with epirubicin and 50 (31%) previously received TACE with cisplatin. Among these patients, the median number of TACE procedures was 4 (range, 1–13), and the median interval between the last previous TACE procedure and miriplatin administration was 4 months (range, 1–41).

Table 1. Characteristics of 162 hepatocellular patients who underwent transcatheter arterial chemotherapy using miriplatin

Variables[a]	Without embolization	With embolization	P value
Number of cases	40	122	
Age (years)	74 (45–91)	72 (45–87)	0.490[b]
Gender (male)	75%	65%	0.230[c]
Albumin (g/dl)	3.3 (2.5–4.2)	3.3 (2–4.2)	0.494[b]
Total bilirubin (mg/dl)	1.1 (0.4–4.7)	1.1 (0.4–4.9)	0.864[b]
Prothrombin (%)	78 (48–100)	83 (45–123)	***0.034***[b]
Platelet ($\times 10^3/\mu l$)	83 (36–261)	93 (29–282)	0.485[b]
Child–Pugh score	6 (5–9)	6 (5–9)	0.197[b]
α-Fetoprotein (ng/ml)	67 (3–331 900)	31 (1.8–152 800)	0.517[b]
Des-γ-carboxyprothrombin (AU/l)	39 (9–4626)	53 (6–65 290)	0.758[b]
Dosage of miriplatin (mg)	75 (20–120)	80 (20–120)	0.981[b]
Dosage of lipiodol (ml)	3 (1–6)	3 (1–6)	0.085[b]
Tumor size (mm)	20 (7–82)	20 (10–100)	0.639[b]
Number of tumors	4 (1–50)	4 (1–100)	0.725[b]
Previous transcatheter arterial chemoembolization	80%	80%	0.946[c]
Injection from segmental branch of the hepatic artery	10%	18%	0.229[c]
Evaluation time point (months)	2.1 (1–3)	2.2 (1–3)	0.758[b]

There were no significant differences between the two groups for any baseline characteristics except for PT.
[a]Variables are expressed as median (minimum–maximum).
[b]Mann–Whitney U-test.
[c]χ^2 test.

The median dosages of miriplatin were 75 mg (range, 20–120 mg) in the TAI group and 80 mg (range, 20–120 mg) in the TACE group ($P = 0.981$). In the TAI group, four patients (10%) were injected with the miriplatin–lipiodol suspension via the peripheral to segmental branch of the hepatic artery. Eleven patients (28%) were injected with the miriplatin–lipiodol suspension via the anterior or posterior segmental branch of the right hepatic artery. Twenty patients (50%) were injected with the miriplatin–lipiodol suspension via the right or left branch of the hepatic artery, and five patients (13%) were injected with the miriplatin–lipiodol suspension via the proper hepatic artery. In the TACE group, 22 patients (18%) were injected with the miriplatin–lipiodol suspension via the peripheral to segmental branch of the hepatic artery. Thirty patients (25%) were injected with the miriplatin–lipiodol suspension via the anterior or posterior segmental branch of the right hepatic artery. Sixty-six patients (54%) were injected with the miriplatin–lipiodol suspension via the right or left branch of the hepatic artery, and four patients (3%) were injected with the miriplatin–lipiodol suspension via the proper hepatic artery.

Treatment Effects

The times of the evaluations after treatment was not statistically significant between the two groups of patients ($P = 0.758$). Forty-one of 162 (25%) patients achieved CR, 42 (25%) achieved PR, 51 (31%) maintained SD and 28 (17%) developed PD. In the TAI group, 6 of 40 (15%) patients achieved CR and 7 of 40 (18%) achieved PR, for an objective response rate of 33%. In the TACE group, 35 of 122 (29%) patients achieved CR and 35 of 122 (29%) patients achieved PR, for an objective response rate of 57%. Although there was no significant difference in the CR rate ($P = 0.084$), the objective response rate was significantly higher in the TACE group than in the TAI group ($P = 0.003$; Table 2).

Among the treatment-naïve patients with HCC, 10 of 32 (31%) patients achieved CR, 12 (38%) achieved PR, 8 (25%) maintained SD and 2 (6%) developed PD. In the TAI group, one of eight (13%) patients achieved CR and two of eight (25%) achieved PR, for an objective response rate of 38%. In the TACE group, 9 of 24 (38%) patients achieved CR and 10 of 24 (42%) patients achieved PR, for an objective response rate of 79%. There was no significant difference in the objective response rates of treatment-naïve patients undergoing TAI versus those undergoing TACE ($P = 0.072$).

Table 2. Tumor response[a] 1–3 months after miriplatin administration

	CR	PR	SD	PD	Total
Number of TAI patients (%)	6 (15%)	7 (18%)	18 (45%)	9 (22%)	40
Number of TACE patients (%)	35 (29%)	35 (29%)	33 (27%)	19 (15%)	122
P value	CR rate, $P = 0.084^b$				
	Objective response rate, $P = 0.003^b$				

TAI, transcatheter arterial infusion chemotherapy; TACE, transcatheter arterial chemoembolization; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
[a]Responses assessed by mRECIST criteria.
[b]*P* values were analyzed by the χ^2 test.

Results of Laboratory Testing

Table 3 shows the results of blood samples tested before, 1 week after and 1 month after miriplatin administration. In the TAI group, the only significant difference seen after miriplatin administration was for AFP concentrations. PT, AFP and DCP values were significantly decreased in the TACE group. For multiple comparisons using Bonferroni adjustments, tumor markers were significantly decreased between

Table 3. Blood samples tested before, 1 week after and 1 month after miriplatin administration

	Pre-treatment	1 week	1 month	*P* value
TAI group ($n = 40$)				
Total bilirubin (mg/dl)	1.1 (0.4–4.7)	1.1 (0.5–4.6)	1.1 (0.4–5.4)	0.710[a]
Albumin (g/dl)	3.3 (2.5–4.2)	–	3.2 (2.6–4.1)	0.640[b]
Prothrombin activity (%)	68.9 (53–99)	73.2 (53–91)	69.1 (55–87)	0.337[a]
Platelet ($\times 10^3/\mu l$)	8.3 (3.6–26.1)	8.4 (2.9–18.5)	9.0 (3.7–22.1)	0.064[a]
AFP ($\mu g/l$)	60.0 (4.3–282 200)	44.5 (4.5–237 200)	63.5 (4.0–331 900)	***0.035***[a]
DCP (AU/l)	39.0 (9–4626)	58.0 (7–4024)	64.0 (10–3540)	0.970[a]
TACE group ($n = 122$)				
Total bilirubin (mg/dl)	1.1 (0.4–4.9)	1.0 (0.2–3.5)	1.0 (0.3–4.0)	0.338[a]
Albumin (g/dl)	3.3 (2.0–4.2)	–	3.3 (2.1–4.4)	0.386[b]
Prothrombin activity (%)	78.5 (53–123)	73.5 (46–100)	76.1 (51–95)	***0.002***[a]
Platelet ($\times 10^3/\mu l$)	10.0 (2.9–28.2)	8.8 (3.2–27.4)	9.6 (2.9–31.7)	0.501[a]
AFP ($\mu g/l$)	34.0 (2.6–36800)	25.8 (1.9–14440)	24.0 (3.0–30890)	***<0.0001***[a]
DCP (AU/l)	55.0 (9–39050)	37.0 (10–14490)	26.0 (6–15518)	***<0.0001***[a]

Values are expressed as median (minimum–maximum). In the TAI group, the only significant difference seen after miriplatin administration was for AFP concentrations. PT, AFP and DCP values were significantly decreased in the TACE group. AFP, α-fetoprotein; DCP, des-γ-carboxyprothrombin.
[a]*P* values were analyzed by the Friedman test.
[b]*P* values were analyzed by the Wilcoxon signed rank test.