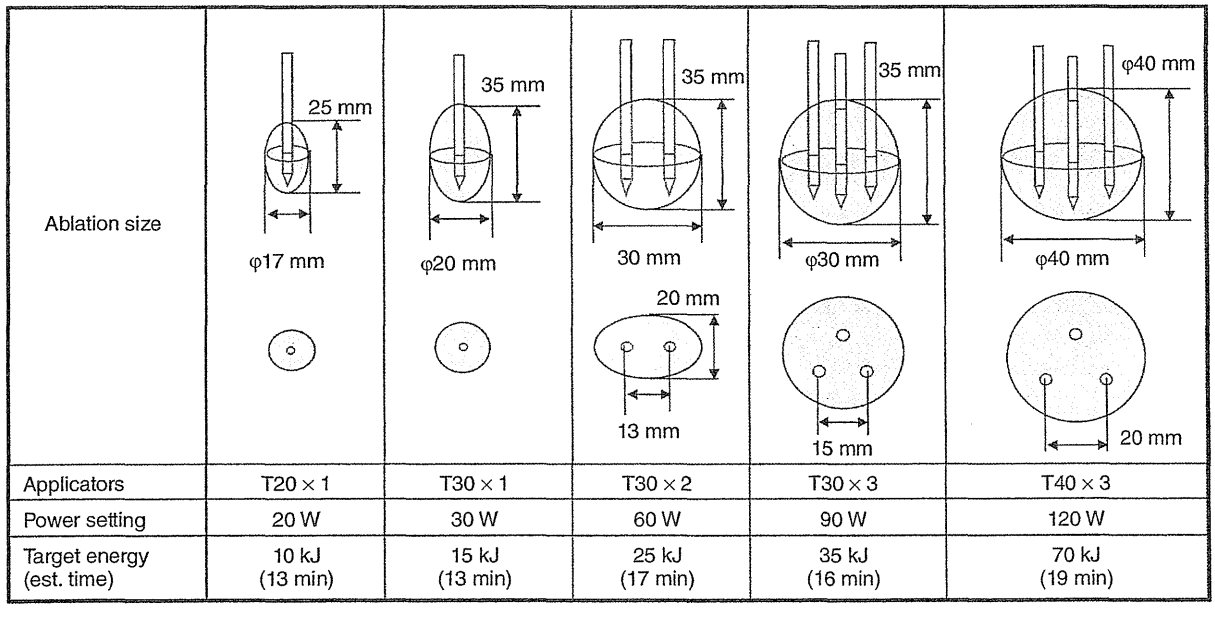
Table 1 Features of each pincer ablation procedure for the treatment of the virtual target located on the liver surface

	Pattern 1			Pattern 2			Pattern 3		*P*
	1	2	3	1	2	3	1	2	
Duration	13′46″	13′16″	12′58″	14′38″	13′50″	13′30″	13′05″	12′40″	$P = 0.151$
Ablated area									
Transverse diameter, mm	27	35	32	25	27	35	45	40	$P = 0.113$
Longitudinal length, mm	35	30	30	32	30	30	27	25	$P = 0.102$
Ablated area covered liver surface	Yes	Yes	Yes	Yes	Yes	Yes	No	No	
Liver surface carbonization appeared	Yes	Yes	Yes	Yes	Yes	Yes	No	No	

protrude from the liver surface. In addition, we investigated only the fan-shape insertion method at a maximum interval of 20–25 mm. The reason for this is that in an actual RFA procedure, it is occasionally difficult to insert two electrodes in the same intercostal space for slightly large nodules that protrude from the liver surface; therefore, in this study, we examined a fan-shape ablation method that assumed two different intercostal approaches. Our results showed that with the pattern 3 treatment procedure, we could not acquire a sufficient ablative margin to the side of the liver surface. From these results, tumors of 20 mm or more may not be suitable for a no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

In contrast, with the pattern 1 and 2 treatment procedures, we acquired a sufficient ablative margin to the side of the liver surface with carbonization of the liver



Applicators	T20 × 1	T30 × 1	T30 × 2	T30 × 3	T40 × 3
Power setting	20 W	30 W	60 W	90 W	120 W
Target energy (est. time)	10 kJ (13 min)	15 kJ (13 min)	25 kJ (17 min)	35 kJ (16 min)	70 kJ (19 min)

- The data are based on Frericks et al., Radiology (2005) 237: 1056–1062. The reported average efficacy was ~0.5 millitre ablation volume per kilojoule.
  From these data, the required energy for an ablation sphere or ellipsoid of given diameter was calculated.
- The application of blood flow interruption (e.g. Pringle's manoeuvre, embolization) allows for a significant reduction of the target energy.

Disclaimer: this dosimetry table does not replace the monitoring of actual ablation sizes. The ablation diameters are approximations based on statistical data; they are not guaranteed for individual clinical cases. Ablation size and shape as well as the procedure time may significantly vary due to tumor physiology and vascular structure. A deviation from the recommended applicator distances may also have an impact on the ablation dimensions.

Figure 3 Dosimetry table for the CelonPOWER system (in Japan).

surface. These results may indicate that tumors of less than 15 mm are candidates for the no-touch pincer ablation procedure that uses two internally cooled bipolar electrodes in this bipolar system.

Finally, this experimental animal study had some limitations. First, the number of animals was very small, and the target tumor was a virtual tumor. Second, an additional examination regarding a no-touch linear insertion procedure for maximum intervals of 20 mm and 25 mm for each electrode was not enforced. Third, we could not investigate the same fan-shape ablation procedure using monopolar RFA in this study, because we assumed it would be too difficult to carry out a two-step insertion method using a monopolar electrode under the influence of a first ablation for nodules that protrude from the liver surface. Fourth, we could not investigate the pathological changes in the ablative area in this study. Therefore, with only these study results, it may not be possible to draw conclusions regarding the utility of the fan-shape insertion method using a bipolar RFA device. To solve these problems, we must carry out an additional large-scale study that includes pathological examination in the near future.

Finally, to summarize the points to be noted at the time of performing the pincer ablation procedure, first, we should insert the needle carefully under US guidance, because in this procedure, measuring the distance of the needle tip from the liver surface and the two needle intervals on the liver surface correctly is the most important point.

Second, with this procedure, we should pay attention to the risk of thermal damage to the visceral peritoneum. Therefore, if possible, thermal protection using measures such as artificial ascites should be considered.

Third, in this study, we did not observe a portal or hepatic vein thrombus in the ablative area. However, this study was performed mainly in the vicinity of the liver surface, and usually this area does not include large vessels. Therefore, we need to use caution as with monopolar ablation when we ablate near large vessels.

In conclusion, the no-touch pincer ablation procedure (with an electrode interval of ≤20 mm) may be useful when performed with two internally cooled bipolar electrodes for small HCC tumors that protrude from the liver surface.

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Original Article

Effectiveness and safety of reduced-dose telaprevir-based triple therapy in chronic hepatitis C patients

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Aim: To compare the early virological effectiveness, sustained virological response and safety of telaprevir 1500 mg/day with telaprevir 2250 mg/day, when combined in triple therapy with pegylated interferon and ribavirin in Japanese patients with high viral loads of genotype 1 hepatitis C virus.

Methods: The telaprevir 2250 mg/day and 1500 mg/day groups each contained 60 patients matched by age, sex and history of previous interferon-based treatment. Serum levels of genotype 1 hepatitis C virus RNA, hemoglobin levels, drug adherence and drug discontinuation rates were monitored during and after triple therapy.

Results: Patients receiving telaprevir 1500 mg/day had significantly lower telaprevir adherence and lower initial ribavirin dose but similar or superior pegylated interferon and ribavirin adherence and a lower rate of telaprevir discontinuation than did those receiving telaprevir 2250 mg/day. The early virological responses and sustained virological response rates were similar in both groups. Hemoglobin levels decreased to a greater extent in patients treated with telaprevir 2250 mg/day.

Conclusion: Compared to triple therapy including telaprevir 2250 mg/day, that including telaprevir at a reduced dose of 1500 mg/day was associated with lower rates of anemia and similar antiviral efficacy. Such a regimen may meaningfully improve sustained virological response rates, especially among female and elderly Japanese patients.

Key words: chronic hepatitis, hepatitis C virus, pegylated interferon, ribavirin, telaprevir

INTRODUCTION

APPROXIMATELY 170 MILLION people are chronically infected with hepatitis C virus (HCV) worldwide,[1] and approximately 30% develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC).[2,3] Currently, interferon (IFN) is the only antiviral drug capable of eliminating HCV infection. The present standard of care (SOC) for patients infected with HCV genotype 1, the most prevalent global genotype, is pegylated interferon (PEG IFN) combined with ribavirin (RBV) for 48 weeks.[4] However, sustained virological response (SVR), defined as the reduction of serum HCV RNA to undetectable levels 24 weeks after the completion of therapy, is achieved in only 42–52% of patients.[5–7] Moreover, response rates are influenced by patient factors such as sex, age and ethnicity,[8–10] as well as virological factors such as genotype and viral load.[11] SVR rates remain unsatisfactorily low (22%) in women aged 50 years or more who are infected with HCV genotype 1 in Japan.[12] Hence, there is a pressing need to improve the efficacy of antiviral treatment in such patients.

Recently, a new class of drugs, with a mechanism based on inhibition of the NS3/NS4 protease of the HCV polyprotein, has been investigated for the treatment of chronic hepatitis C. Of the drugs in this class, telaprevir has been selected as a clinical candidate for further development.[13] Telaprevir combined with PEG IFN and RBV has shown potent antiviral activity in phase II[14,15] and III clinical trials;[16,17] SVR rates of

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approximately 70% have been reported in patients infected with HCV-1. Similarly, in Japan, a phase III study was conducted in patients with HCV-1 to compare the efficacy and safety of the telaprevir regimen with those of the current SOC in treatment-naïve patients,[18] and to assess the efficacy and safety of the telaprevir regimen in relapsers and non-responders after previous IFN-based therapy.[19] However, the high efficacy was offset by treatment-induced anemia: early hemoglobin levels during triple therapy decreased by up to 4 g/dL, whereas decreases with SOC were not higher than 3.0 g/dL.[14,15] Additionally, we have previously reported that the factors associated with decreases in hemoglobin levels during triple therapy included female sex and age of more than 50 years.[20] Japanese patients infected with HCV genotype 1b with high viral loads are, on average, much older than Western patients infected with the same genotype, owing to a widespread HCV infection that occurred in Japan approximately 20 years ago.[21] Therefore, we considered that triple therapy would be highly effective when combined with careful monitoring of hemoglobin levels and prompt modification of RBV dose.

Consequently, in this study, we evaluated the effectiveness and safety of telaprevir-based triple therapy, administrated at an initial telaprevir dose of 2250 or 1500 mg/day, in the retrospective matched control study of 120 Japanese patients with chronic HCV-1 infection with high viral loads.

METHODS

Patients

FROM DECEMBER 2008 to August 2012, 204 patients with chronic hepatitis C were recruited to receive triple therapy with telaprevir, PEG IFN and RBV for 24 weeks at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. All patients had the following characteristics: (i) positive for HCV RNA genotype 1 and antibody to HCV (anti-HCV), absence of co-infection with HCV of other genotypes; (ii) negative for hepatitis B surface antigen; (iii) HCV RNA levels of 5.0 log IU/mL or more as determined with the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (iv) platelet counts of more than $80 \times 10^3/mm^3$ without cirrhosis diagnosed by ultrasonography; (v) not pregnant or lactating; (vi) total previous alcohol intake of less than 500 kg; (vii) absence of HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; and (viii) absence of antiviral or immunosuppressive treatment during the previous 3 months.

Patients were followed for liver function and virological markers at least monthly during treatment and until 24 weeks after completion of the triple therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the a priori approval of the institution's human research committee.

Study design

Telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) was administrated at the dose of 2250 (750 mg three times daily) or 1500 mg/day (750 mg twice daily). We selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment from the telaprevir 2250 and 1500 mg/day groups (Table 1), because 204 patients had many differences in baseline characteristics in both groups. PEG IFN-α-2b (PEG-Intron; Schering Plough, Kenilworth, NJ, USA) was injected s.c. at a median dose of 1.5 μg/kg (range, 1.1–1.8) once a week. RBV (Rebetol; Schering Plough) was administrated at 200–1000 mg/day; RBV dose of 600 mg/day (for bodyweight ≤60 kg), 800 mg/day (for bodyweight >60 to ≤80 kg) or 1000 mg/day (for bodyweight >80 kg) in principle. Since November 2011, the initial dose of RBV was reduced by 200 mg in cases of female sex, aged 66 years or older, hemoglobin level of less than 13 g/dL, bodyweight of less than 45 kg or platelet counts of less than $150 \times 10^3/mm^3$ at baseline by the judgment of the physician. All participating patients received these three drugs for the initial 12 weeks, followed by PEG IFN and RBV for an additional 12 weeks. All patients were followed up for at least 24 weeks after the last dose of study drugs to assess SVR.

Doses of telaprevir, PEG IFN and RBV were reduced or their administration discontinued as required, based on the reduction of hemoglobin levels; reduction of white blood cell, neutrophil or platelet counts; or the development of adverse events. Thus, the total dose of each drug administrated during the 12–24 weeks was calculated as the ratio of the actual administrated total dose to the anticipated total dose of each drug; these ratios provided adherence measures for telaprevir, PEG IFN and RBV.

HCV RNA measurements

Blood samples were obtained at weeks 1, 2, 4, 6, 8, 12, 16, 20 and 24 after initiation of treatment and at week 24 after completion of treatment, and routine biochemical

Table 1 Baseline characteristics of the patients infected with genotype 1 HCV who received triple therapy with pegylated interferon, ribavirin and TVR

	TVR 2250 mg/day	TVR 1500 mg/day	*P*-value
n	60	60	
Sex (male/female)	30/30	30/30	Matched
Age (years)	60 (53–63)	62 (56–64)	Matched
Body mass index (kg/m^2)	22.1 (20.4–24.0)	22.7 (20.1–24.8)	0.278
IL28B genotype (rs8099917) TT/TG + GG	40/20	54/6	0.003
ITPA genotype (rs12979860) CC/CA + AA	44/16	36/23	0.175
Hemoglobin (g/dL)	14.3 (13.5–15.2)	14.2 (13.0–14.8)	0.223
Platelets (×104/μL)	17.6 (14.9–21.0)	16.9 (13.8–19.9)	0.227
Albumin (g/dL)	3.8 (3.7–4.0)	3.8 (3.7–4.1)	0.404
Alanine aminotransferase (IU/L)	35 (25–49)	37 (25–58)	0.437
γ-Glutamyltransferase (IU/L)	29 (18–49)	22 (17–39)	0.230
Creatinine (mg/dL)	0.7 (0.6–0.8)	0.6 (0.6–0.7)	0.333
Uric acid (mg/dL)	5.6 (4.9–6.5)	5.5 (4.7–6.3)	0.487
α-Fetoprotein (μg/L)	4 (3–7)	5 (3–8)	0.740
HCV RNA (log10 IU/mL)	6.8 (6.4–7.0)	6.7 (6.3–7.0)	0.551
Core a.a. 70 (wild/mutant)	38/22	45/15	0.235
Core a.a. 91 (wild/mutant)	28/32	36/24	0.200
Previous IFN-based treatment			
Naïve/relapsed/null response	23/25/12	23/25/12	Matched

Values are number with percentage in parentheses or median with interquartile range in parentheses.
a.a., amino acid; HCV, hepatitis C virus; IFN, interferon; TVR, telaprevir.

and hematological tests were performed. The antiviral effects were assessed by measuring plasma HCV RNA levels using the COBAS TaqMan HCV test. The linear dynamic range of the assay was 1.2–7.8 $\log_{10}$ IU/mL; undetectable samples were defined as negative.

Detection of amino acid substitutions in the core of HCV-1b

Amino acid (a.a.) substitutions in the HCV core region were determined using direct sequencing of polymerase chain reaction products after extraction and reverse transcription of HCV RNA. Core a.a. substitutions at positions 70 and 91 (core 70 and 91, respectively) were determined according to the methods of our previous reports.[22,23]

Determination of IL28B and ITPA genotypes

ITPA (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped using the Invader assay, TaqMan assay or direct sequencing, as described.[24,25]

Statistical analyses

Non-parametric tests, including the χ^2-test, Fisher's exact test, Mann–Whitney *U*-test and Kruskal–Wallis tests, were used to analyze differences in the baseline clinical profiles of patients. Kaplan–Meier analysis and the log-rank test were applied to estimate and compare serum HCV RNA elimination rates between the groups. $P < 0.05$ by two-tailed test was considered statistically significant. All analyses were performed using SPSS software version 10.1 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics

THE BASELINE CHARACTERISTICS of the 120 patients are listed in Table 1. There were no significant differences in the baseline characteristics between the telaprevir 2250 mg/day group and 1500 mg/day group, except for *IL28B* genotypes. Patients receiving telaprevir 1500 mg/day had a significantly higher incidence of TT in *IL28B* genotypes than did those receiving 2250 mg/day.

Initial drug doses, drug adherence and discontinuation rate up to 12 weeks

Patients receiving telaprevir 1500 mg/day had a significantly lower initial telaprevir dose and initial RBV dose than those receiving 2250 mg/day (Table 2). Telaprevir adherence was significantly lower in the 1500 mg/day

Table 2 Initial drug doses, drug adherence up to 24 weeks and discontinuation rates up to 12 weeks

	TVR 2250 mg/day	TVR 1500 mg/day	*P*-value
n	60	60	
Initial TVR dose (mg/kg per day)	38.1 (33.6–45.1)	25.6 (22.5–29.6)	<0.001
TVR adherence up to 12 weeks (%)	100 (75–100)	67 (65–67)	<0.001
Discontinuation of TVR	15 (25.0%)	6 (10.0%)	0.053
Discontinuation of TVR due to anemia	12 (20%)	3 (5%)	0.025
Initial PEG IFN dose (μg/kg per week)	1.5 (1.4–1.6)	1.5 (1.4–1.6)	0.706
PEG IFN adherence up to 24 weeks (%)	100 (85–100)	100 (89–100)	0.062
Initial RBV dose (mg/kg per day)	11.6 (10.6–12.8)	9.9 (7.9–11.3)	<0.001
RBV adherence up to 24 weeks (%)	51 (41–61)	59 (46–68)	0.090
Discontinuation of all drugs up to 12 weeks	5 (8.3%)	1 (1.7%)	0.207

Values are number with percentage in parentheses or median with interquartile range in parentheses.
PEG IFN, pegylated interferon; RBV, ribavirin; TVR, telaprevir.

group than in the 2250 mg/day group, while there were no differences in adherence for the other two drugs. Although there were no significant differences between the groups in the rates of discontinuation of telaprevir or all drugs up to 12 weeks, the rates of discontinuation of telaprevir due to anemia in the 1500 mg/day group were significantly lower than in 2250 mg/day group.

Loss of serum HCV RNA according to *IL28B* genotypes

Figure 1 compares the on-treatment virological response over the first 12 weeks for the telaprevir 2250 and 1500 mg/day groups according to *IL28B* genotypes, respectively, because there were significant differences in distribution of *IL28B* genotypes between both groups.

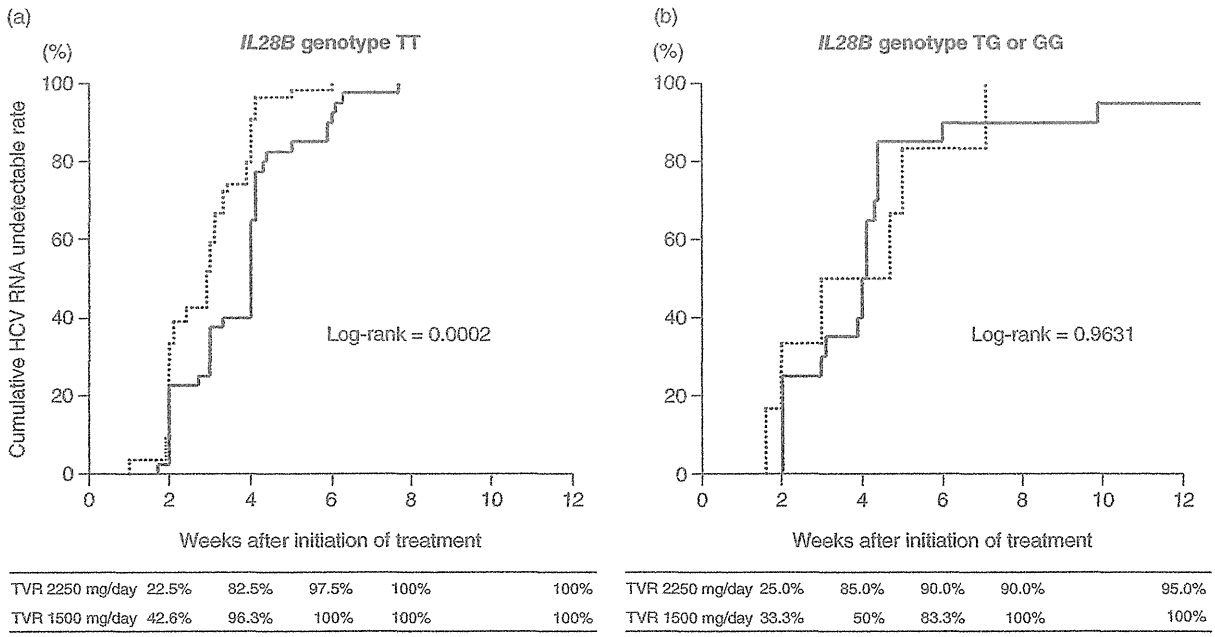

Figure 1 Cumulative rate of undetectable hepatitis C virus (HCV) RNA during triple therapy with pegylated interferon, ribavirin and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. (a) *IL28B* genotype TT, (b) *IL28B* genotype TG or GG. (———) TVR 2250 mg/day, (·····) TVR 1500 mg/day.

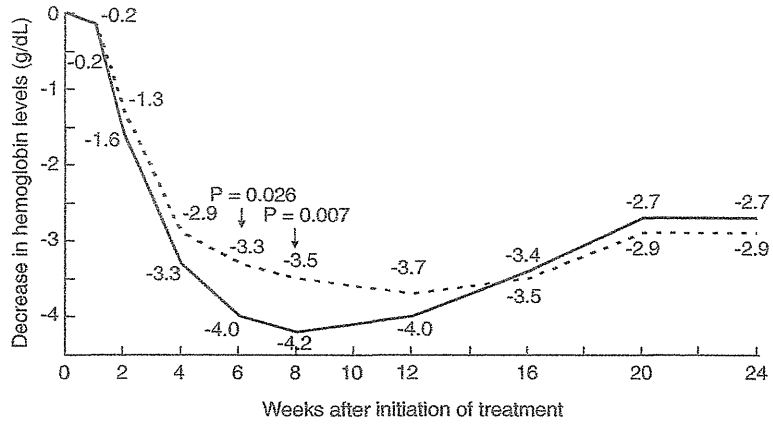

Figure 2 Decreases in hemoglobin levels during triple therapy with pegylated interferon (PEG IFN), ribavirin (RBV) and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. Each time point in this figure corresponds to median values. Patients evaluated at each time point are indicated below, with the number of patients who discontinued TVR (continued PEG IFN and RBV) in parentheses. (———) TVR 2250 mg/day, (·····) TVR 1500 mg/day.

Number of patients (TVR withdrawn)								
2250 mg/day	60	60	60 (1)	59 (4)	55 (10)	55	55	55
1500 mg/day	60	60	60 (1)	59 (2)	59 (3)	59	59	59

Triple therapy suppressed HCV RNA levels quickly and effectively in both groups. In the 2250 and 1500 mg/day groups of *IL28B* genotype TT, HCV RNA became undetectable in 22.5% and 42.6% of patients at 2 weeks, 82.5% and 96.3% at 4 weeks, and 100% and 100% at 8 weeks, respectively (Fig. 1a). The early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group in *IL28B* genotype TT (log-rank test = 0.0002).

In the subgroups of *IL28B* genotype non-TT patients receiving telaprevir 2250 and 1500 mg/day, HCV RNA became undetectable in 25.0% and 33.3% of patients at 2 weeks, 85.0% and 50% at 4 weeks, 90.0% and 100% at 8 weeks, and 95.0% and 100% at 12 weeks, respectively. The virological responses during the first 12 weeks in this subgroup of patients did not significantly differ between the telaprevir 2250 and 1500 mg/day groups (log-rank test = 0.9631, Fig. 1b).

Safety

Figure 2 shows the decreases in hemoglobin levels in telaprevir 2250 and 1500 mg/day recipients. Data from six patients were omitted (five receiving telaprevir 2250 mg/day and one receiving 1500 mg/day) because treatment was withdrawn between 8 and 12 weeks after initiation. Telaprevir was discontinued in 15 of the 60 (25.0%) patients receiving telaprevir 2250 mg/day (one at week 6, four at week 8 and 10 at week 12) and six of the 60 (10.0%) receiving 1500 mg/day (one at week 6, two at week 8 and three at week 12). Hemoglobin decreased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 6 (−4.0 [−6.7 to −1.2] vs −3.3 [−5.2 to 0.2] g/dL, $P = 0.026$) and week 8 (−4.2 [−7.7 to −1.3] vs −3.5 [−6.9 to −1.3] g/dL, $P = 0.007$).

Skin disorder frequency was comparable between the telaprevir 2250 mg/day group and 1500 mg/day group (81.7% and 75%, respectively). However, skin disorders of grades 2–3 occurred more frequently in the telaprevir 2250 mg/day group than in the 1500 mg/day group (55% vs 35%, $P = 0.043$).

With respect to renal dysfunction, increases in serum creatinine (sCR) levels during therapy were not significantly different between both groups. However, blood uric acid levels increased to a greater extent in patients receiving telaprevir 2250 mg/day than in those receiving 1500 mg/day at week 1 (1.3 [−1.6 to 4.8] vs 0.9 [−2.1 to 4.3] g/dL, $P = 0.015$), week 2 (1.2 [−2.3 to 4.1] vs 0.5 [−2.3 to 2.7] g/dL, $P = 0.004$), week 4 (1.6 [−1.1 to 5.5] vs 0.7 [−2.4 to 3.8] g/dL, $P < 0.001$), week 6 (1.6 [−1.7 to 4.8] vs 0.5 [−3.5 to 3.6] g/dL, $P < 0.001$) and week 8 (1.1 [−3.6 to −4.9] vs 0.7 [−1.6 to 3.7] g/dL, $P = 0.029$).

Predictive factors associated with SVR

The overall SVR rate was 83% (169/204) in our hospital. SVR was accomplished in 106 (88%) of 120 patients selected for this study, including 50 of 60 (83%) patients in the telaprevir 2250 mg/day and 56 of 60 (93%) patients in telaprevir 1500 mg/day groups (Fig. 3).

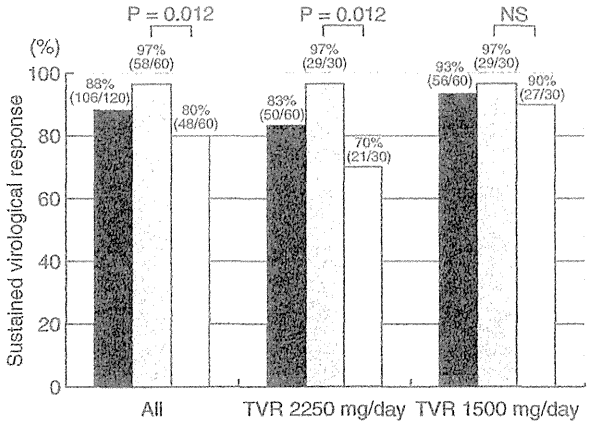

Figure 3 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared among all patients (men and women), TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (■) Total, (□) male, (□) female.

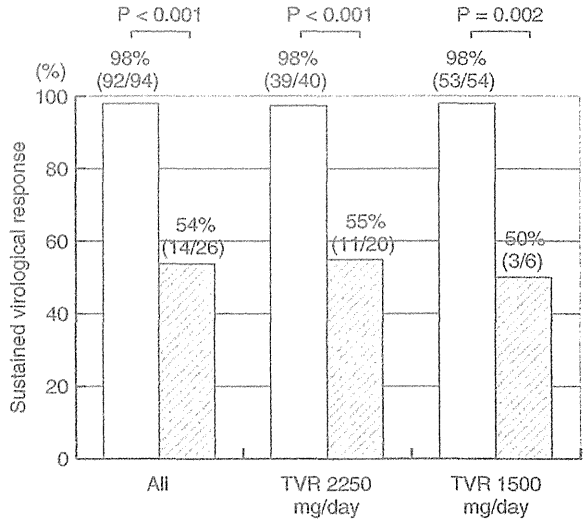

Figure 4 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared between *IL28B* (rs8099917) genotype TT and TG/GG in all patients, TVR 2250 mg/day patients and TVR 1500 mg/day patients, respectively. (□) TT, (□) TG or GG.

Significant univariate predictors for SVR included male sex, *IL28B* genotype TT, and HCV core a.a. 70 wild type, except for null response to prior treatment, initial telaprevir dose of 37.5 mg/kg per day or more, telaprevir dosing period of 10 weeks or more, 100% PEG IFN adherence up to 24 weeks, PEG IFN adherence up to 12 weeks of 80% or more, RBV adherence up to 12 weeks of 50% of more, γ-glutamyltransferase of 35 IU/mL or less, and sCr of 0.6 mg/dL or more ($P < 0.05$). Of these, male sex (odds ratio [OR] = 13.7; $P = 0.028$) and *IL28B* genotype TT (OR = 44.4; $P = 4.47 \times 10^{-5}$) were identified as significant independent predictors for SVR (Table 3).

Therefore, we assessed the SVR rate of triple therapy according to sex and *IL28B* genotype. SVR was much less frequent in women than in men (48/60 [80%] vs 58/60 [97%], $P = 0.0012$, Fig. 3). Especially, in the telaprevir 2250 mg/day group, there were significant differences between men and women (29/30 [97%] vs 21/30 [70%], $P = 0.0012$). However, there were no differences between men and women in the telaprevir 1500 mg/day group (29/30 [97%] and 27/30 [90%], respectively).

Patients with *IL28B* genotype TT were significantly more likely to achieve SVR (92/94 [98%] vs 14/26 [54%], $P < 0.001$, Fig. 4), compared with patients with TG or GG genotypes. There were significant differences between *IL28B* genotype TT and non-TT in both the telaprevir 2250 and 1500 mg/day groups (39/40 [98%] vs 11/20 [55%], $P < 0.001$ and 53/54 [98%] vs 3/6 [50%], $P = 0.002$, respectively).

Table 3 Multivariate analysis of factors associated with sustained virological response of TVR, pegylated interferon and ribavirin triple therapy in Japanese patients infected with HCV

Factor	Category	Odds ratio (95% CI)	*P*-value
Sex	1; female	1	
	2; male	13.7 (1.33–141.2)	0.028
IL28B genotype (rs8099917)	1; TG or GG	1	
	2; TT	44.4 (7.18–274.2)	4.47×10^{-5}

CI, confidence interval; HCV, hepatitis C virus; TVR, telaprevir.

DISCUSSION

IN JAPANESE PATIENTS, virological response to triple therapy with telaprevir, PEG IFN and RBV was excellent. We have previously reported that in 20 patients with chronic HCV-1b infection with high viral load who received triple therapy for 12 weeks, HCV RNA became undetectable in 50% at 2 weeks, 79% at 4 weeks, 88% at 6 weeks, 94% at 8 weeks and 100% at 12 weeks.[26] This previous study was a randomized open-label study in which telaprevir was administrated at doses of 2250 or 1500 mg/day. Early virological response at 7 and 14 days was similar for both telaprevir doses, suggesting that virological response to triple therapy is not affected by lowering the telaprevir dose. Therefore, to expand the dataset, we retrospectively evaluated HCV RNA response and safety during 12 weeks of triple therapy including the two different telaprevir doses followed by PEG IFN and RBV for an additional 12 weeks: we analyzed 204 cases in total. However, because of the non-random nature of treatment allocation, there was a preponderance of women, elderly and anemic patients in the group receiving telaprevir 1500 mg/day. Because there were many differences in baseline characteristics between telaprevir 2250 and 1500 mg/day groups, we selected 60 patients per group who were matched by age, sex and history of previous IFN-based treatment. Therefore, there were no differences in baseline characteristics between both groups in this analysis, except for *IL28B* genotype. Although we tried to match the distribution of *IL28B* genotypes between both groups, this was not possible because of the small number of cases. Therefore, we matched the groups by the history of previous IFN-based treatment, which we considered a similarly strong predictive factor of triple therapy. Moreover, there was a significant difference in the initial dose of RBV between both groups. A significant number of patients underwent RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group because we considered that such patients were likely to experience hemoglobin decrements during triple therapy, but before November 2011, we could not reduce the initial dose of telaprevir and RBV. Nine patients (15.0%) receiving telaprevir 2250 mg/day and 32 cases (53.3%) receiving 1500 mg/day underwent RBV dose reduction at the beginning of treatment. In other words, the group receiving telaprevir 1500 mg/day had a significantly lower initial dose of telaprevir and RBV dose than did the group receiving 2250 mg/day (Table 2).

However, in the present study, HCV RNA became undetectable during the 12 weeks of treatment at similar or higher rates in the telaprevir 1500 mg/day group than in the 2250 mg/day group (Fig. 1). In the *IL28B* TT genotype, the early virological response of the telaprevir 1500 mg/day group was significantly higher than that of the 2250 mg/day group. Although we assessed baseline factors, drug adherence and drug discontinuation rates only in the *IL28B* TT genotype, there were no significant differences between both groups, except for lower telaprevir adherence up to 12 weeks and a greater number of cases of PEG IFN and RBV dose reductions at the beginning of treatment in the telaprevir 1500 mg/day group. Therefore, the reason for significant differences in the early virological response between both groups is unclear. However, we considered that these results did not affect the SVR rate because HCV RNA became undetectable in all patients in both groups at 8 weeks after the start of triple therapy. In all cases, *IL28B* TT cases and non-TT cases, there were no significant differences in SVR rates after triple therapy between those receiving telaprevir 2250 and 1500 mg/day (Figs 3,4). By examining the detailed course of drug administration from 12–24 weeks (Table 2), we found that the group receiving telaprevir 1500 mg/day had a lower discontinuation rate of telaprevir and higher adherence to RBV and PEG IFN up to 24 weeks in spite of the low initial RBV dose. Furthermore, hemoglobin levels showed greater reductions during triple therapy with telaprevir 2250 mg/day than with telaprevir 1500 mg/day, and the group receiving telaprevir 2250 mg/day had a significantly higher discontinuation rate of telaprevir due to anemia than did the group receiving telaprevir 1500 mg/day (Fig. 2). Therefore, telaprevir 1500 mg/day may be a safe option as part of triple therapy, while maintaining PEG IFN and RBV adherence.

Viral breakthrough or relapse can occur during telaprevir monotherapy or telaprevir plus PEG IFN dual therapy (without RBV) because of the development of mutations that confer resistance to telaprevir.[14,27–29] Furthermore, in a Japanese phase III trial of triple therapy in relapsers and non-responders who had not achieved SVR to a previously administrated IFN-based regimen, SVR rates increased as RBV adherence increased, particularly in previous non-responders.[19] In triple therapy with telaprevir, PEG IFN and RBV, we consider that telaprevir could be important for early virological response, but it could also be important for maintaining high adherence to PEG IFN and RBV, which is a key factor for achieving SVR. We speculate that triple therapy including telaprevir at the reduced dose of 1500 mg/day could maintain high levels of adherence

to PEG IFN and RBV, and consequently achieve high SVR rates.

In this study, we investigated the independent predictors for SVR in the multivariate analysis (Table 3). As reported in previous studies, *IL28B* genotype remained the strongest predictor of SVR.[30,31] The next strongest predictive factor was sex: women had significantly lower SVR rates than did men (Fig. 3). However, when we investigated the SVR rates of the telaprevir 2250 mg/day group and 1500 mg/day group, we found that there were significant differences in SVR rates between men and women in the telaprevir 2250 mg/day group but no differences in the telaprevir 1500 mg/day group. In the previous study, we reported that female sex was one of the factors influencing decreases in hemoglobin levels during triple therapy administrated 2250 mg/day of initial telaprevir dose.[20] In the present study, the discontinuation rates of telaprevir due to anemia were significantly higher in women in the telaprevir 2250 mg/day group as compared with men (36.7% vs 3.3%, $P = 0.002$, data not shown), but there were no differences in the discontinuation rates of telaprevir due to anemia between men and women in the telaprevir 1500 mg/day group (0% vs 10%, $P = 0.237$, data not shown). Therefore, we speculate that there were significant differences in SVR rates between men and women because of high telaprevir discontinuation rates owing to anemia in women.

In conclusion, after the completion of 24 weeks of therapy, triple therapy including telaprevir at a reduced dose of 1500 mg/day was as effective as triple therapy including telaprevir 2250 mg/day at suppressing HCV RNA to undetectable levels and achieving SVR. Of note, we found that telaprevir 1500 mg/day was associated with lower levels of anemia and discontinuation of telaprevir owing to anemia, and higher PEG IFN and RBV adherence during triple therapy. These results suggest that the telaprevir 1500 mg/day regimen is an effective and safe alternative for the treatment of elderly and female Japanese patients. This study is a retrospective study. Prospective randomized controlled studies with longer follow-up periods are required to fully assess the efficacy and safety of an initial telaprevir dose of 1500 mg/day.

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ORIGINAL PAPER

The safety of chemotherapy for colorectal cancer patients with hepatitis C virus infection

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Abstract Hepatitis C virus (HCV) infection is one of the most common blood-borne infections worldwide. Little is known with respect to changes in HCV status during chemotherapy for colorectal cancer (CRC), and the influence of HCV infection on chemotherapy remains unclear. Between 2001 and 2012, 3,260 patients were diagnosed with CRC in our institute. We studied 77 patients who were positive for anti-HCV antibodies. We retrospectively reviewed changes in HCV load and chemotherapy toxicities. Twenty-four of 77 HCV-infected patients with CRC received chemotherapy. Their median age was 66 years, and four patients had liver cirrhosis. The remaining 20 patients were diagnosed with chronic hepatitis, and their liver function tests and blood cell counts at baseline were normal. Serum HCV ribonucleic acid level before and after chemotherapy was evaluated in ten patients, with medians of 4.0 and 3.05 log IU/ml at baseline before and after chemotherapy, respectively. Two patients demonstrated elevated transaminase levels during chemotherapy. Among the 24 HCV patients received chemotherapy, no patients suffered from febrile neutropenia or treatment delay; two required chemotherapy dose reduction. Our results indicated that chemotherapy for CRC patients with HCV infection can be performed safely without changing the viral load.

Keywords Chemotherapy · Colorectal cancer · Hepatitis C infection · Reactivation · Viral load

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Introduction

Hepatitis C virus (HCV) infection is one of the most common blood-borne infections worldwide and is a major cause of chronic liver disease in Japan, with an estimated infected population of >880,000 people [1]. Colorectal cancer (CRC) is one of the most commonly diagnosed malignancies in Japan, affecting over 100,000 individuals. Systemic chemotherapy for patients with CRC is now accepted as a standard therapy to improve outcomes.

Little is known with respect to changes in the HCV load during chemotherapy for CRC, and the influence of HCV infection on chemotherapy toxicity remains unclear. Although reactivation of hepatitis B virus is well documented in patients receiving chemotherapy, and management guidelines exist, the effect of chemotherapy on patients with HCV is not well described [2, 3]. Coppola et al. [4] evaluated changes in HCV ribonucleic acid (RNA) both in plasma and peripheral blood mononuclear cells in patients with onco-hematological disease who received rituximab and combination chemotherapy and noted increased HCV load during chemotherapy. However, there are substantial differences in the immunosuppressive mechanisms between rituximab-based chemotherapy for hematological malignancies and chemotherapy for solid tumor. Therefore, it may not be appropriate to use the same management strategy during chemotherapy for HCV-carrier patients with solid tumors.

The aim of this study was to evaluate the safety profile and changes in HCV viral load during chemotherapy for HCV-carrier patients with CRC.

Patients and methods

This study was approved by the Institutional Review Board of Toranomon hospital.

Patient selection

We performed a retrospective survey of 3,260 patients diagnosed with CRC between 2001 and 2012 at our institution. All of the patients were screened for hepatitis C serology by anti-HCV antibody at the time of CRC diagnosis. Seventy-seven patients were serologically positive for anti-HCV antibodies, and 24 of 77 patients received chemotherapy. We retrospectively analyzed these 24 patients' baseline characteristics, clinicopathological data of the tumor characteristics, changes in HCV load, and the chemotherapy toxicities, based on a review of their medical records.

Assessment of HCV infection status

HCV-antibody testing was performed using chemiluminescence enzyme immunoassay (Lumipulse Ortho HCV antigen, Ortho-Clinical Diagnostics, Tokyo, Japan) at the time of CRC diagnosis. HCV RNA in serum was detected using a TaqMan real-time polymerase chain reaction assay (SRL, Tokyo, Japan). In ten patients, serum HCV RNA was evaluated before the initiation of chemotherapy and within 8 weeks after completing chemotherapy.

Assessment of liver function

Liver cirrhosis was assessed based on clinical criteria (Child–Pugh criteria) at baseline. We measured the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin at baseline, and prothrombin time during chemotherapy and after completion of chemotherapy. Adverse events, evaluated by the Common Terminology Criteria for Adverse Events version 4.0, were also retrospectively collected.

Assessment of other toxicities

White blood cell count and platelet count at baseline and during chemotherapy were collected and assessed using Common Terminology Criteria for Adverse Events version 4.0. We recorded febrile neutropenic events and the use of growth factor support during chemotherapy.

Results

Patients' characteristics

Patients' and tumor characteristics are shown in Table 1. The median age of the patients when receiving chemotherapy was 66 years (range, 42–80 years). Most patients (58 %) had stage III disease, and the tumor histology of all patients was adenocarcinoma. Five patients had a diagnosis of liver cirrhosis: two patients were classified as Child B, and the remaining three patients were Child A. Five patients showed elevated levels of HCV RNA, and no patient had decompensated liver disease before chemotherapy.

Treatment course and safety

Table 2 shows the chemotherapy regimens and their associated documented toxicities. First-line chemotherapy included 5-fluorouracil/leucovorin and oxaliplatin ($N = 6$), S-1 and irinotecan ($N = 1$), uracil/tegafur ($N = 14$), capecitabine ($N = 1$), and capecitabine and oxaliplatin ($N = 2$). Transaminases were elevated to grade 1 or 2 in ten (42 %) patients and to grade 3 in two (8 %) patients for whom chemotherapy was discontinued because of liver dysfunction. In these two patients, one patient developed Child A liver cirrhosis and the other patient developed Child B liver cirrhosis. The transaminase levels before and after chemotherapy in the patient who developed Child A liver dysfunction were 93/94 IU/L (AST/ALT) and 206/69 IU/L, respectively. The transaminase level in the patient who developed Child B liver dysfunction increased from 41/26 IU/L (AST/ALT) to 301/194 IU/L during chemotherapy.

Eight patients developed grade 1–2 thrombocytopenia, and three developed grade 3. Three patients developed grade 2 neutropenia, and three developed grade 3–4. Granulocyte colony stimulating factor was not used in any patient, and no febrile neutropenia was observed. Two patients required chemotherapy dose reduction because of hematological toxicities.

HCV RNA status

Table 3 shows the changes in HCV RNA in ten patients. The median HCV RNA level before the initiation of chemotherapy was 5.5 and 5.05 log IU/ml after completing chemotherapy. HCV RNA level was not elevated in the two patients who had grade 3 transaminase elevation during chemotherapy.

Table 1 Baseline characteristics of the patients

Variables	Patients in the study ($n = 24$)
Age	66 (42–80)
Male	13
Female	11
Stage	
I	1
IIA	6
IIB	1
IIIA	1
IIIB	9
IIIC	4
IV	2
Histrogy	
tub1	11
tub2	12
por	1
Location	
Cecum	2
Ascending colon	1
Transverse colon	3
Descending colon	1
Sigmoid colon	7
Rs	5
Ra	4
Rb	1
C–P score	
Class A	22
Class B	2
LC	
Positive	4
Negative	20
Prior HCV therapy	
Yes	4
No	20

Case	Age	Stage	Histology	Tumor location	Child–pugh	LC	Prior HCV therapy
1	47	T2N2M0 (Stage IIIC)	tub2	C	5:A	Negative	Negative
2	80	T3N1M0 (Stage IIIB)	por	Ra	5:A	Negative	Negative
3	52	T3N2M0 (Stage IIIC)	tub2	Rs	5:A	Positive	Positive
4	60	T2N2M0 (Stage IIIC)	tub1	S	6:A	Negative	Negative
5	72	T4N0M1 (Stage IV)	tub2	Rs	5:A	Negative	Negative
6	78	T3N0M0 (IIA)	tub2	S	5:A	Negative	Negative
7	70	T3N2M0 (Stage IIIC)	tub2	Ra	8:B	Positive	Negative
8	61	T3N1M0 (Stage IIIB)	tub2	Rs	7:B	Positive	Positive
9	51	T3N0M0 (IIA)	tub2	Ra	5:A	Negative	Negative
10	76	T3N1M0 (Stage IIIB)	tub1	Rs	6:A	Positive	Positive
11	75	T2N0M0 (Stage I)	tub1	T	6:A	Negative	Negative
12	42	T4N0M0 (Stage IIB)	tub2	D	5:A	Negative	Negative
13	49	T3N0M0 (IIA)	tub2	Ra	5:A	Positive	Positive
14	64	T3N1M0 (Stage IIIB)	tub2	S	5:A	Negative	Negative

Table 1 continued

Case	Age	Stage	Histology	Tumor location	Child–pugh	LC	Prior HCV therapy
15	68	T3N1M0 (Stage IIIB)	tubl	S	6:A	Negative	Negative
16	71	T3N1M0 (Stage IIIB)	tubl	S	5:A	Negative	Negative
17	70	T3N1M0 (Stage IIIB)	tubl	S	6:A	Negative	Negative
18	61	T3N3M1 (Stage IV)	tubl	Rs	6:A	Negative	Negative
19	69	T2N1M0 (Stage IIIA)	tubl	T	5:A	Negative	Negative
20	51	T3N0M0 (IIA)	tubl	T	5:A	Negative	Negative
21	63	T3N0M0 (IIA)	tub2	Rb	6:A	Negative	Negative
22	79	T3N1M0 (Stage IIIB)	tubl	C	5:A	Negative	Negative
23	58	T3N0M0 (IIA)	tubl	S	5:A	Negative	Negative
24	74	T3N1M0 (Stage IIIB)	tub2	A	5:A	Negative	Negative

tubl Tubular adenocarcinoma, well-differentiated, *tub2* tubular adenocarcinoma, moderately differentiated, *por* poorly differentiated adenocarcinoma, *C* cecum, *A* ascending colon, *T* transverse colon, *D* descending colon, *S* sigmoid colon, *R* rectum, *LC* liver cirrhosis, *HCV* hepatitis C virus

Discussion

Our findings revealed that chemotherapy for CRC patients with HCV infection is feasible and that viral load does not elevate during chemotherapy. Reactivation of hepatitis B virus is well documented in patients receiving chemotherapy, especially in allogenic bone marrow transplantation or other forms of immunosuppression. However, the effect of such therapies on patients with HCV is not well described. [5].Reactivation in patients with solid tumors treated with chemotherapy is rare, especially in patients with gastrointestinal cancer. Melisko et al. [6] reported the clinical course of HCV reactivation in a patient receiving chemotherapy for metastatic colon cancer, and de Pree et al. [7] described a patient with esophageal cancer receiving radiochemotherapy who developed an acute exacerbation of HCV infection. A small number of retrospective studies on breast cancer chemotherapy in HCV-infected patients have shown good safety and tolerability. Shoji et al. [8] reported that approximately 80–90 % of breast cancer patients with HCV could complete chemotherapy without experiencing grade 3 or 4 leukopenia, thrombocytopenia, or liver enzyme elevation.

Previous studies have reported chemotherapy-induced transaminase elevation in patients with HCV infection [6, 9]; however, the relationship between increases in HCV RNA and transaminase elevation is poorly investigated. Morrow et al. [10] showed that nine of 36 (25 %) HCV-positive breast cancer patients who received chemotherapy developed liver enzyme elevation, but their study did not evaluate HCV load. Shoji et al. [8] also reported that 16 of 52 (30.8 %) HCV-positive breast cancer patients who received chemotherapy developed hepatitis, and two of the 16 patients showed elevated levels of HCV RNA [8]. Melisko et al. [6] reported a patient who suffered reactivation of HCV after the fourth cycle of chemotherapy with bolus irinotecan, 5-fluorouracil and folic acid. However, they did not screen for HCV antibody or HCV RNA before chemotherapy and also transfused with two units of packed red blood cells after colectomy; therefore, it is not certain whether the HCV RNA elevation was caused by the reactivation or the influence of acute HCV infection related to the blood transfusion. To our knowledge, our study is the first report of HCV RNA measurement before and after chemotherapy in CRC patients with HCV infection.

We found no clinically meaningful changes in HCV RNA viral load in CRC patients who received oxaliplatin, irinotecan, uracil/tegafur, capecitabine, or capecitabine and oxaliplatin. Only two patients (8 %) had grade 3 transaminase elevation during chemotherapy, and their HCV RNA level was not elevated before or after chemotherapy. These findings suggest that the transaminase elevation in our study might not have been related to HCV viral reactivation but instead, to direct liver toxicity from cytotoxic agents.

Some studies have suggested that B cell-mediated immunosuppression induced by rituximab results in transaminase elevation [4, 9, 11]. Coppola et al. [4] showed that rituximab-based chemotherapy increases the HCV viral load in anti-HCV-positive onco-hematological patients, likely by reducing the CD20 lympho-monocytic cell numbers and activity, leading to a reduction in the immune control on HCV replication. However, the mechanisms of liver injury in HCV infection are still unknown. Further investigations into the relationship between HCV load and liver injury are warranted.

In conclusion, our analysis indicated that chemotherapy for CRC patients with HCV infection is feasible and should not be withheld owing to positive HCV serology. However, our study is too small to draw a definite conclusion on the

Table 2 Regimens and toxicities for the HCV patients

Chemotherapy regimen	HCV patients ($n = 24$)
UFT	14
FOLFOX	6
XELOX	2
IRIS	1
Capecitabine	1
Transaminase baseline	
Increase	6
Normal	18
Transaminase increase (grade)	
Grade 0	12
Grade 1	9
Grade 2	1
Grade 3	2
PT baseline	
Normal	24
PT decrease after Cx	0
WBC baseline	
Normal	23
WBC decrease after Cx	6
Platelet baseline	
Decrease	4
Normal	20
Thrombocytopnia G1-3	11
Febrile neutropenia	0
Use G-CSF	0
Dose reduction	2

Case	Chemotherapy regimen	Transaminase baseline (grade)	Transaminase increase (grade)	WBC Baseline (grade)	WBC decrease (grade)	Neutropenia baseline (grade)	Neutropenia decrease (grade)	Plt Baseline (grade)	Thrombocytopenia (grade)	G-CSF	FN	dose reduction (%)
1	FOLFOX	Increase (1)	1	decreased (1)	1	2	3	Normal	1	None	0	None
2	FOLFOX	Normal	0	Normal	0	0	0	Normal	0	None	0	None
3	FOLFOX	Normal	1	Normal	2	0	4	Normal	3	None	0	None
4	FOLFOX	Normal	1	Normal	0	0	0	Normal	0	None	0	None
5	FOLFOX	Normal	0	Normal	2	0	2	Normal	0	None	0	80
6	FOLFOX	Normal	0	Normal	0	0	0	Normal	0	None	0	None

Table 2 continued

Case	Chemotherapy regimen	Transaminase baseline (grade)	Transaminase increase (grade)	WBC Baseline (grade)	WBC decrease (grade)	Neutropenia baseline (grade)	Neutropenia decrease (grade)	Plt Baseline (grade)	Thrombocytopenia (grade)	G-CSF	FN	dose reduction (%)
7	IRIS	Normal	0	Normal	0	0	0	Decrease (1)	1	None	0	None
8	UFT	Increase (1)	3	Normal	0	0	0	Normal	0	None	0	None
9	UFT	Normal	0	Normal	0	0	0	Normal	0	None	0	None
10	UFT	Normal	1	Normal	0	0	0	Normal	0	None	0	None
11	UFT	N/A	0	Normal	0	0	0	Normal	0	None	0	None
12	UFT	Normal	0	Normal	0	0	0	Normal	0	None	0	None
13	UFT	Normal	0	Normal	0	0	0	Normal	0	None	0	None
14	UFT	Normal	1	Normal	1	0	2	Normal	1	None	0	None
15	UFT	Increase (1)	1	Normal	1	0	0	Decrease (1)	1	None	0	None
16	UFT, FOLFOX	Normal	1	Normal	0	0	0	Normal	1	None	0	None
17	UFT, FOLFOX	Normal	0	Normal	0	0	0	Normal	1	None	0	None
18	CapeOX	Normal	1	Normal	0	0	0	Normal	1	None	0	None
19	Capecitabine	Increase (1)	2	Normal	0	0	0	Normal	0	None	0	None
20	UFT	Normal	0	Normal	0	0	0	Normal	0	None	0	None
21	UFT	Increase (1)	1	Normal	0	0	0	Decrease (1)	2	None	0	None
22	UFT	Increase (1)	0	Normal	0	1	2	Decrease (1)	3	None	0	None
23	CapeOX	Normal	0	Normal	1	0	3	Normal	3	None	0	80
24	UFT	Increase	3	Normal	0	0	0	Normal	0	None	0	None

Table 3 HCV RNA viral load (log IU/ml)

Case	baseline	After chemotherapy
1	6.6	6.1
2	Normal range	Normal range
3	4.1	3.2
4	6.4	5.7
5	Normal range	Normal range
6	Normal range	Normal range
7	5.1	5.7
8	6.3	6.3
9	Normal range	Normal range
10	5.5	4.1

safety of chemotherapy in patients with HCV infection. Further large-scale investigations are needed to clarify the associations between HCV reactivation and chemotherapy.

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Original Article

Discrimination of fibrotic staging of chronic hepatitis C using multiple fibrotic markers

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Aim: In order to evaluate and judge a fibrotic stage of patients with chronic hepatitis C, multivariate regression analysis was performed using multiple fibrotic markers.

Methods: A total of 581 patients from eight hepatology units and institutes were diagnosed by needle biopsy as having chronic liver disease caused by hepatitis C virus. Twenty-three variables and their natural logarithmic transformation were employed in the multivariate analysis.

Results: Multivariate regression analysis finally obtained the following function: $z = 2.89 \times ln$ (type IV collagen 7S) (ng/mL) $- 0.011 \times$ (platelet count) ($\times 10^3/mm^3$) $+ 0.79 \times ln$ (total bilirubin) (mg/dL) $+ 0.39 \times ln$ (hyaluronic acid) (μg/L) $- 1.87$. Median values of the fibrotic score of F1 ($n = 172$), F2 ($n = 80$), F3 ($n = 37$) and F4 ($n = 16$) were calculated as 1.00, 1.45, 2.82 and 3.83, respectively. Multiple regression coefficient and coefficient of determination were 0.56 and 0.320, respectively. Validation with patient data from other institutions demonstrated good reproducibility of the fibrotic score for hepatitis C (FSC), showing 1.10 in F1 ($n = 156$), 2.35 in F2 ($n = 73$), 3.16 in F3 ($n = 36$) and 3.58 in F4 ($n = 11$).

Conclusion: A concise multiple regression function using four laboratory parameters successfully predicted pathological fibrotic stage of patients with hepatitis C virus infection.

Key words: chronic hepatitis, hepatitis C virus, liver cirrhosis, liver fibrosis, multiple regression analysis, stage

INTRODUCTION

WHEN HEPATITIS C virus (HCV)-related chronic liver disease was found by biochemical and virological examination, peritoneoscopy and/or liver biopsy can establish the definitive diagnosis of chronic hepatitis and liver cirrhosis. Although these pathological procedures are reliable and informative both in diagnosis and treatment, they sometimes require medical invasion and financial costs, including the risk of bleeding from needle puncture, some pain experienced during the examination, medical expenses and hospitalization for a few days. The pathological examination is, therefore, rarely performed repeatedly in a short period of time, even when disease activity is severe and progression of liver disease is highly suspected. Recently, many authors described the usefulness of ultrasonographic elastography and magnetic resonance imaging technology in the estimation of staging of chronic hepatitis and cirrhosis.[1–4] These ways of estimation using the imaging apparatuses seem truly useful for current patients, but it cannot evaluate and compare with past fibrotic states of patients retrospectively. Moreover, the same apparatus for elastometry will not be available for repeated measurement for a follow-up examination, several years later for example.

In spite of the accuracy of biopsy and of convenience of elastography in chronic liver disease, clinical diagnosis based on biochemistry and hematology is still indispensable for the daily practice of many patients with

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HCV-related liver disease. Recently, several studies were published about estimation of hepatitis stages, using one or more serum biomarkers. Discriminant functions or multivariate analyses demonstrated that approximately 60–90% of patients with chronic hepatitis C were correctly classified as mild hepatitis and severe hepatitis with advanced fibrosis.[5–16] The usefulness of the discriminant functions was, however, less valuable up to the present time for a few reasons. First, these functions were made for the purpose of discrimination of severe hepatic fibrosis from mild fibrosis, and four histological classifications (F1, F2, F3 and F4) were selected in almost of the studies. Second, some studies analyzed both hepatitis B virus and HCV infection, although the significance and actual values of each liver function test in the evaluation of the severity of liver disease were not similar among each viral hepatitis and alcoholic liver disease. Third, biochemical markers for liver fibrosis (e.g. hyaluronic acid, type IV collagen, procollagen III peptide)[17–19] were not always included in those previous studies.

We tried to generate a function estimating fibrotic stages of HCV-related chronic hepatitis, which were objectively diagnosed by liver biopsy. The purpose of this study is, therefore, to make a reliable multiple regression function and to obtain practical coefficients for significant variables also using fibrotic markers.

METHODS

Patients

A TOTAL OF 605 Japanese patients with chronic hepatitis C were recruited for the study from eight hospitals in Japan: Toranomon Hospital, Hiroshima University Hospital (K. Chayama, M.D.), Ehime University Hospital (M. Onji, M.D.), Musashino Red Cross Hospital (N. Izumi, M.D.), Shishu University Hospital (E. Tanaka, M.D.), Showa University Hospital (M. Imawari, M.D.), Osaka University Hospital (T. Takehara, M.D.) and Kagoshima University Hospital (H. Tsubouchi, M.D.). Inclusion criteria for this study were: (i) positive HCV antibody for more than 6 months; (ii) persistent or intermittent elevation in aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels; and (iii) liver biopsy showing chronic hepatitis (F1, F2, F3 or F4). We excluded those patients with overt alcoholic liver disease or fatty liver, association of other types of liver disease (e.g. hepatitis B, primary biliary cirrhosis, autoimmune hepatitis), or those associated with hepatocellular carcinoma or other malignancy. Among the patients, 603 fulfilled the conditions for the study: complete demographic data, basic laboratory data of hematology and biochemistry, required liver biopsy specimens, and sufficient amount of frozen sera. We also excluded an additional 22 patients with eventual histological diagnosis of F0 stage.

Finally, a total of 581 patients who were diagnosed as having chronic hepatitis or cirrhosis (F1, F2, F3 or F4) were analyzed for the following hematological, biochemical and histopathological examination. There were 305 males and 276 females aged 15–78 with a median of 55 years.

All the patients presented written informed consent in individual hospitals and medical centers, and the study was approved by each ethical committee.

Hematological and biochemical examination

Hematological and standard biochemical evaluation had been performed in each medical institution: white blood cell, red blood cell count, hemoglobin, platelet count, total bilirubin, AST, ALT, AST/ALT ratio (AAR), γ-glutamyltransferase (GGT), total protein, albumin and γ-globulin.

Special biochemical examinations including fibrotic markers were carried out using stored frozen sera at −20°C or lower: α2-macroglobulin, haptoglobin concentration, haptoglobin typing, apolipoprotein A1, hyaluronic acid, tissue inhibitor of matrix metalloproteinase (TIMP)-1, TIMP-2, procollagen III peptide and type IV collagen 7S.

Histological diagnosis of chronic hepatitis and cirrhosis

All of the 581 cases fulfilled required standards of histological evaluation: sufficient length of specimen, hematoxylin–eosin staining and at least one specimen with fiber staining. Four independent pathologists (Y. T., J. F., F. K. and T. F.), who were not informed of patients' background and laboratory features except for age and sex, evaluated the 581 specimens regarding the stages of fibrosis and activity. Pathological classification of chronic hepatitis staging was based on Desmet *et al.*[20]

Before judgment of histological staging of individual specimens, the pathologists discussed objective and reproducible judgment of pathological diagnosis of hepatitis. They made a panel for obvious criteria using typical microscopic pictures for each stage, and it was always referred to during the procedure of pathological judgment. When inconsistent results were found in the diagnosis of stage of hepatitis among the pathologists, the final judgment was accepted as the majority rule among them.