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Original Article

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

Hitomi Sezaki, Fumitaka Suzuki, Tetsuya Hosaka, Norio Akuta, Taito Fukushima, Tasuku Hara, Yusuke Kawamura, Masahiro Kobayashi, Yoshiyuki Suzuki, Satoshi Saitoh, Yasuji Arase, Kenji Ikeda and Hiromitsu Kumada

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

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

Correspondence: Dr Hitomi Sezaki, Department of Hepatology, Toranomon Hospital, Minato-ku, Tokyo 105-8470, Japan. Email: hitomis@mx1.harmonix.ne.jp

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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

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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

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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.

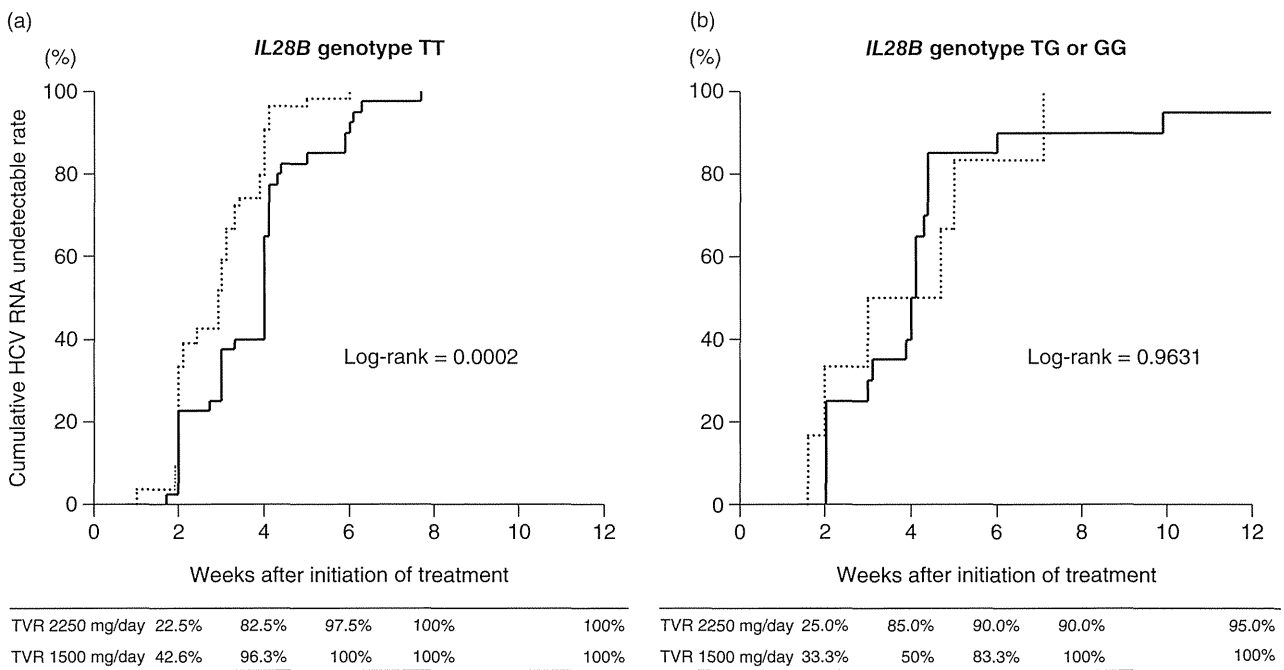

Figure 1 Cumulative rate of undetectable hepatitis C virus (HCV) RNA during triple therapy with pegylated interferon, ribavirin and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. (a) *IL28B* genotype TT, (b) *IL28B* genotype TG or GG. (———) TVR 2250 mg/day, (·····) TVR 1500 mg/day.

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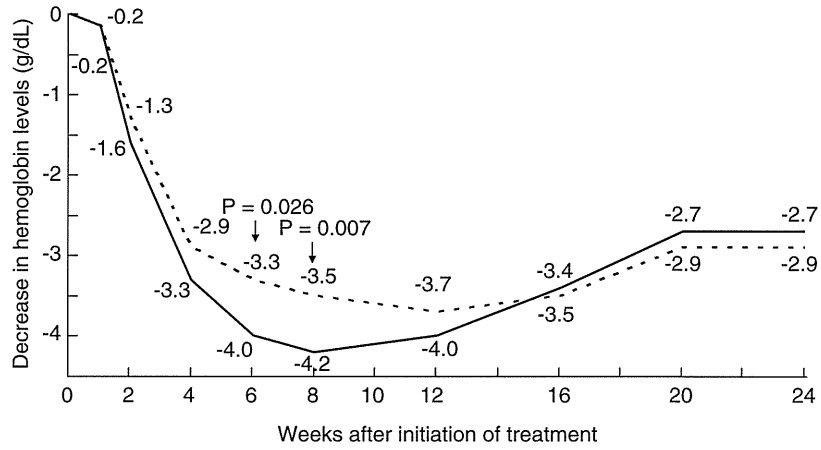

Figure 2 Decreases in hemoglobin levels during triple therapy with pegylated interferon (PEG IFN), ribavirin (RBV) and telaprevir (TVR) at either 2250 mg/day or 1500 mg/day. Each time point in this figure corresponds to median values. Patients evaluated at each time point are indicated below, with the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

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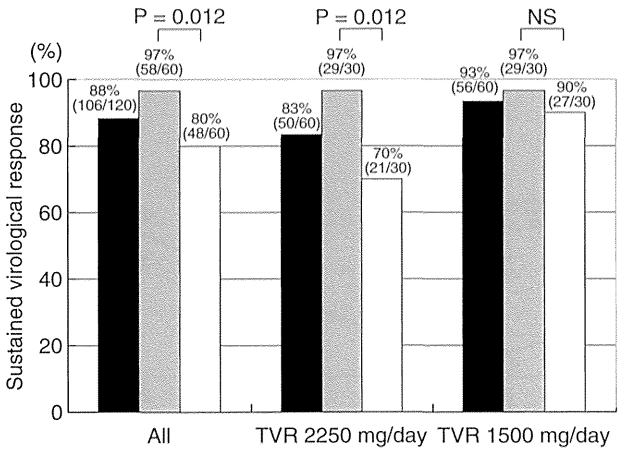

Figure 3 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared among all patients (men and women), TVR 2250 mg/day patients and TVR 1500 mg/day patients,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

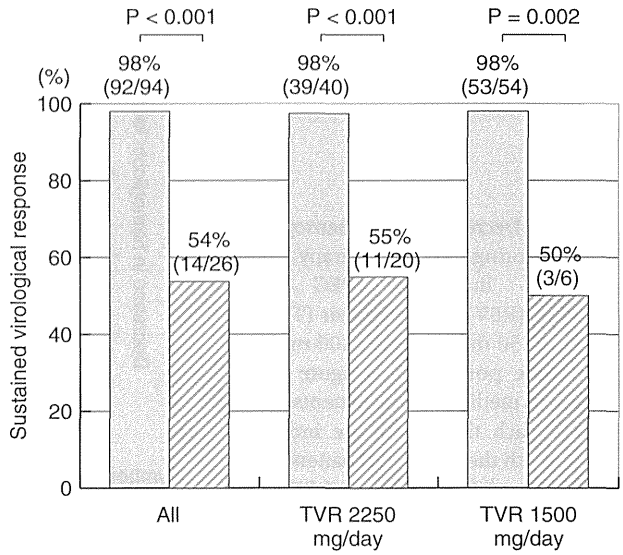

Figure 4 Sustained virological response in patients with chronic hepatitis C to triple therapy with telaprevir (TVR), pegylated interferon and ribavirin for 24 weeks. Sustained virological response was compared between *IL28B* (rs8099917) genotype TT and TG/GG in all patients, TVR 2250 mg/day patients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

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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

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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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Impact of Virus Clearance for the Development of Hemorrhagic Stroke in Chronic Hepatitis C

Yasuji Arase,[1,2,3]* Mariko Kobayashi,[1] Yusuke Kawamura,[1] Fumitaka Suzuki,[1] Yoshiyuki Suzuki,[1] Norio Akuta,[1] Masahiro Kobayashi,[1] Hitomi Sezaki,[1] Satoshi Saito,[1] Tetsuya Hosaka,[1] Kenji Ikeda,[1] Hiromitsu Kumada,[1] and Tetsuro Kobayashi[3]

[1]*Department of Hepatology and Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan*
[2]*Department of Health Management Center, Toranomon Hospital, Tokyo, Japan*
[3]*Department of Third Internal Medicine, University of Yamanashi, Yamanashi, Japan*

The aim of this retrospective cohort study was to assess the cumulative incidence and predictive factors for intracerebral hemorrhagic stroke after the termination of interferon (IFN) therapy in Japanese patients with hepatitis C virus (HCV). A total of 4,649 HCV-positive patients treated with IFN were enrolled. The primary goal is the first onset of intracerebral hemorrhagic stroke. The mean observation period was 8.0 years. Evaluation was performed using the Kaplan–Meier method and the Cox proportional hazard model. A *P*-value of less than 0.05 was considered statistically significant. A total of 28 developed intracerebral hemorrhagic stroke. The cumulative incidence of intracerebral hemorrhagic stroke was 0.3% at 5 years, 0.8% at 10 years, and 1.7% at 15 years. Intracerebral hemorrhagic stroke occurred when patients had age increments of 10 years (hazard ratio: 2.77; 95% confidence interval (CI) 1.48–5.18; $P=0.001$), hypertension (hazard ratio: 2.30; 95% CI 1.09–4.83; $P=0.021$), liver cirrhosis (hazard ratio: 4.50; 95% CI 2.07–9.78; $P<0.001$), and HCV non-clearance (hazard ratio: 3.22; 95% CI 1.22–8.53; $P=0.018$). On the intracerebral hemorrhagic stroke based on the difference of liver fibrosis and efficacy of IFN therapy, HCV clearance reduced to 24.3% (1/4.11) compared to HCV non-clearance in cirrhotic patients ($P=0.040$). In conclusion, HCV clearance reduced the development of intracerebral hemorrhagic stroke. In particular, HCV clearance reduced intracerebral hemorrhagic stroke to about one-fourth in cirrhotic patients. ***J. Med. Virol. 86:169–175, 2014.***

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KEY WORDS: hepatitis C virus; interferon therapy; hemorrhagic stroke

INTRODUCTION

There are 170 million people affected with chronic hepatitis C virus (HCV) infection worldwide, which may cause an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20–50% of cases over a period of 10–30 years [Kiyosawa and Furuta, 1991; Alter et al., 1992]. In addition, HCV is a major risk for hepatocellular carcinoma (HCC) [Hasan et al., 1990; Kew et al., 1990; Ikeda et al., 1993; Tsukuma et al., 1993; Arase et al., 2012]. In addition, several authors have reported that HCV clearance decreases the rate of fibrosis progression and the development of HCC in patients with chronic HCV infection [Kasahara et al., 1998; Yoshida et al., 2002; Arase et al., 2013].

On the other hand, hemorrhagic stroke is a medical emergency and can cause permanent neurological damage and death [Truelsen et al., 2003; Iso et al., 2007; Donnan et al., 2008]. It is becoming a great health burden in most countries. However, there is a little information on the incidence and risk factors on the incidence of hemorrhagic stroke in HCV patients treated with interferon (IFN). Furthermore, it is not clear whether the HCV clearance is useful for

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; CT, computed tomography; GGT, gamma-glutamyltransferase; HbA_{1C}, hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon; LDL, low density lipoprotein

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*Correspondence to: Yasuji Arase, MD, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo 105-8470, Japan.
E-mail: es9y-ars@asahi-net.or.jp

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reducing the development of hemorrhagic stroke in HCV patients.

With this background in mind, the present retrospective cohort study was initiated to investigate the cumulative incidence and risk factors of cerebral stroke after prolonged follow-up in HCV patients treated with IFN. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

PATIENTS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and treated for the first time with IFN monotherapy or combination therapy between September 1990 and May 2010 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan was 7,635. Of these, 4,649 patients satisfied with the following enrolled criteria: (1) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy within 1 year before the initiation of IFN therapy; (2) positivity for serum HCV-RNA before the initiation of IFN therapy; (3) period of $\geq$1 month to $\leq$1 year of IFN therapy; (4) negativity for hepatitis B surface antigens (HBsAg), antibody to hepatitis B core, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence assay; (5) age of $\geq$30 to $\leq$80 years; and (6) no autoimmune systemic disease, such as systemic lupus erythematosus or rheumatic arthritis. Patients with either of the following criteria were excluded from the study: (1) they had illnesses that could seriously reduce their life expectancy; (2) they had a history of coronary and/or cerebrovascular disease; (3) they had a history of carcinogenesis; and (4) they had been given anticoagulant and antiplatelet drugs.

The primary outcome is the first development of hemorrhagic stroke. Hemorrhagic stroke was regarded as intracerebral hemorrhagic stroke in the present study. Thus, patients with subarachnoid hemorrhagic stroke or subdural hematoma were excluded from analyses. The development of hemorrhagic stroke was diagnosed by clinical symptoms and imaging (computed tomography and/or magnetic resonance imaging) based on the World Health Organization definition [Truelsen et al., 2003; Iso et al., 2007; Donnan et al., 2008]. All of the studies were performed retrospectively by collecting and analyzing data from the patient records. The physicians in charge explained the purpose, method, and side effect of IFN therapy to each patient and/or patients' family. In addition, the physicians in charge got permission of serum stores and future uses of stored serum. Informed consent for IFN therapy and future uses of stored serum was obtained from all patients. This study had been approved by Institutional Review Board of our hospital.

Medical Evaluation

Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline, and the body mass index (BMI) was calculated as kg/m^2. All patients were interviewed by physicians or nurse staff in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and health-related habits including questions on alcohol intake and smoking history.

Hemoglobin A_{1C} (HbA_{1C}) was estimated as National Glycohemoglobin Standardization Program equivalent value (%) and fasting plasma glucose [American Diabetes Association, 2010]. Patients were defined as having type 2 diabetes mellitus when HbA_{1C} level was $\geq$6.5% and/or fasting plasma glucose level was $\geq$126 mg/dl. Patients were defined as hypertensive when blood pressure was $\geq$140/90 mmHg or pharmacological treatment for high blood pressure was given. Smoking index (package per day $\times$ year) and total alcohol intake were evaluated by the sum of before, during, and after the IFN therapy.

Laboratory Investigation

Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Anti-HCV was detected using an enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-genotype was examined via polymerase chain reaction assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported [Dusheiko et al., 1994]. HCV-RNA was determined by the COBAS TaqMan HCV test (Roche Diagnostics, Basel, Switzerland). The serum samples stored at -80°C before IFN therapy were used. The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative. A HCV clearance was defined as clearance of HCV RNA using the COBAS TaqMan HCV test 6 months after the cessation of IFN therapy.

Evaluation of Liver Cirrhosis

Status of liver was mainly determined on the basis of peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas [Desmet et al., 1994].

Follow-Up

The observation starting point was 6 months after the termination of IFN therapy. After that, patients were followed up at least twice a year in our hospital.

Biochemical tests were conducted at each examination together with regular check-up. Four hundred fifty patients were lost to follow-up. The final date of follow-up in 452 patients with loss of follow-up was regarded as last consulting day.

Patients with either of the following criteria during follow-up were regarded as censored data in statistical analysis [Fleming et al., 1984]: (1) they were retreated with IFN (N = 949); (2) they had new onset of carcinogenesis (N = 645); and (3) they had been given anticoagulant and antiplatelet drugs (N = 28). The final date of follow-up in these patients with censored data was regarded as the time of the initiation of criteria described above. The mean follow-up period was 6.7 [standard deviation (SD) 4.3] years in 452 patients with loss of follow-up and 7.4 (SD 4.7) years in 1,722 patients who had censored data. Patients with loss of follow-up and censored data were counted in the analysis.

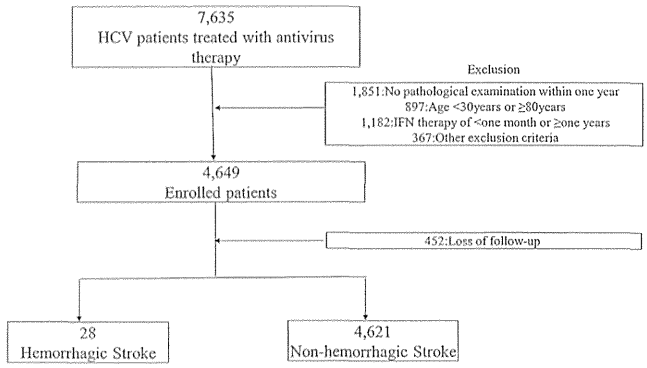


Fig. 1. An algorithm of the study population.

Statistical Analysis

Clinical differences between patients with hemorrhagic stroke and those without events were evaluated using Mann–Whitney test. The cumulative incidence of hemorrhagic stroke were calculated by using the Kaplan–Meier technique, and differences in the curves were tested using the log-rank test [Kaplan and Meier, 1958; Harrington and Fleming, 1983]. Independent risk factors associated with hemorrhagic stroke were studied using the stepwise Cox regression analysis [Cox, 1972]. The following

TABLE I. Clinical Backgrounds at the Initiation of Follow-Up in Enrolled Patients

	Total	Hemorrhagic stroke group	Without events group	*P*-value
N	4,649	28	4,621	
Age (years)	51.9 ± 11.8	60.4 ± 6.7	51.8 ± 11.9	<0.001
Gender (M/F)	2,966/1,883	16/12	2,950/1,871	0.781
Height (cm)	163.1 ± 9.2	159.5 ± 9.4	163.2 ± 9.2	0.171
Weight (kg)	61.4 ± 12.8	57.9 ± 8.0	61.4 ± 12.7	0.113
BMI	22.7 ± 3.1	23.4 ± 2.8	22.7 ± 3.1	0.582
BP (systolic, mmHg)	128 ± 18	140 ± 20	127 ± 18	0.007
BP (diastolic, mmHg)	77 ± 13	86 ± 15	77 ± 13	0.001
Total alcohol intake (kg)[a]	95 ± 92	148 ± 105	94 ± 92	0.002
Smoking index[a]	6.5 ± 9.5	11.8 ± 12.4	6.4 ± 9.4	<0.001
AST (IU/L)	41 ± 43	48 ± 28	41 ± 43	<0.001
ALT (IU/L)	44 ± 53	53 ± 38	43 ± 52	0.004
GGT (IU/L)	53 ± 60	59 ± 47	52 ± 61	0.078
Albumin (g/dl)	4.0 ± 0.3	3.5 ± 0.4	4.0 ± 0.3	0.110
Triglyceride (mg/dl)	101 ± 52	108 ± 46	100 ± 52	0.097
Cholesterol (mg/dl)	170 ± 31	171 ± 27	170 ± 31	0.893
HDL-C (mg/dl)	48 ± 14	45 ± 12	48 ± 14	0.002
LDL-C (mg/dl)	104 ± 29	108 ± 37	103 ± 29	0.049
Fasting plasma glucose (mg/dl)	99 ± 22	103 ± 23	100 ± 22	0.093
HbA_{1C} (%)	5.7 ± 1.1	5.9 ± 1.2	5.7 ± 1.1	0.024
Platelet ($\times 10^4/mm^3$)	17.2 ± 5.2	14.1 ± 6.2	17.3 ± 5.4	0.001
Staging (cirrhosis/non-cirrhosis)[b]	485/4,164	12/16	473/4,148	<0.001
HCV genotype (1b/2a/2b/other)[b]	2,859/1,109/497/184	22/5/1/0	2,837/1,104/496/184	0.104
HCV RNA (log IU/ml)[b]	6.07 ± 1.05	6.03 ± 1.03	6.08 ± 1.05	0.387
IFN monotherapy/combination therapy[c]	3,000/1,649	24/4	2,976/1,645	<0.001
Efficacy (HCV; clearance/non-clearance)	2,103/2,546	5/23	2,098/2,523	0.006

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}; hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.
Data are number of patients or mean ± standard deviation.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.
[b]Value before IFN treatment.
[c]Outbreak of IFN monotherapy: recombinant IFN alpha 2a, 238 cases; recombinant IFN alpha 2b, 183 cases; natural IFN alpha, 1,750 cases; natural IFN beta, 750 cases; total dose of IFN = 554 ± 164 MU. Outbreak of peg IFN monotherapy: peg IFN alpha 2a, 93 cases, total dose of peg IFN = 7.54 ± 2.20 mg.
Outbreak of combination therapy: recombinant IFN alpha 2b + ribavirin, 335 cases, total dose of IFN = 508 ± 184 MU, total dose of ribavirin = 160 ± 68 g; natural IFN beta + ribavirin, 127 cases, total dose of IFN = 502 ± 177 MU, total dose of ribavirin = 155 ± 67 g; peg IFN alpha 2b + ribavirin, 1,173 cases, total dose of peg IFN = 4.12 ± 1.10 mg, total dose of ribavirin = 205 ± 58 g.

variables were analyzed for potential covariates for incidence of primary outcome: (1) age, gender, type 2 diabetes mellitus, hypertension, BMI at the initiation time of follow-up, (2) HCV genotype, HCV load, and hepatic fibrosis before IFN therapy, (3) average value of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, and platelet during follow-up, (4) sum value of smoking and alcohol before, during, and after the IFN therapy, (5) efficacy of IFN therapy, combination of ribavirin, type of IFN, and total dose of IFN. A P-value of less than 0.05 was considered statistically significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

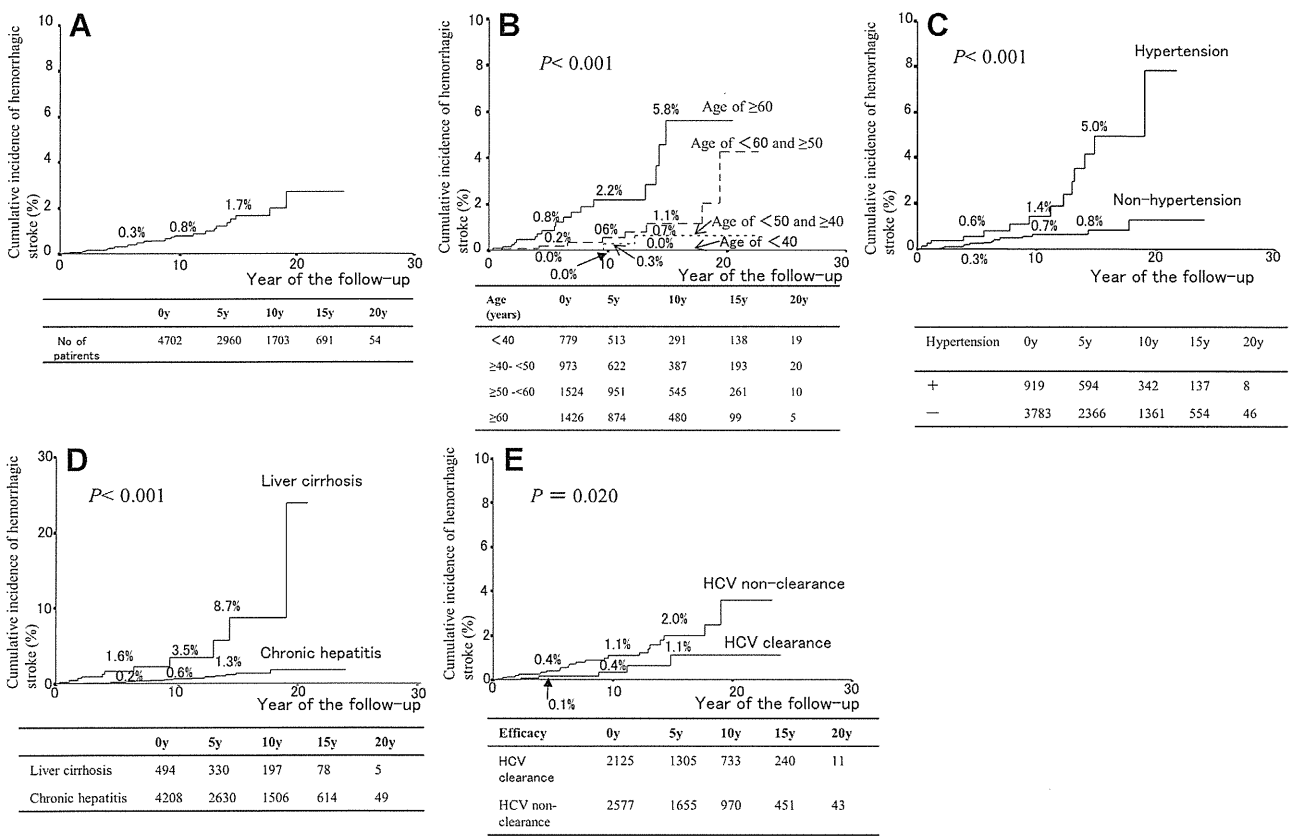
RESULTS

Patients Characteristics

Figure 1 shows the algorithm of the study population. For the mean observation period of 8.0 years, 28 of 4,649 patients developed hemorrhagic stroke. Table I shows the baseline characteristics of the enrolled 4,649 patients at the initiation of follow-up. The patients are divided into two groups of patients with hemorrhagic stroke and without event. There are significant differences in several baseline characteristics between the two groups. The HCV clearance rate was 34.7% (1,042/3,000) in IFN monotherapy and 64.3% (1,061/1,649) in combination therapy of IFN and ribavirin. Thus, the number of patients with HCV clearance was 2,103. The mean follow-up was 8.0 (SD 5.0) years. The 28-day vascular disease-related mortality rate was 33% (10/28) in hemorrhagic stroke.

Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

The cumulative incidence of intracerebral hemorrhagic stroke was 0.3% at 5 years, 0.8% at 10 years, and 1.7% at 15 years (Fig. 2A). The factors associated with the development of intracerebral hemorrhagic stroke are shown in Table II. Intracerebral hemorrhagic stroke occurred when patients had age increments of 10 years [hazard ratio: 2.77; 95% confidence interval (CI) 1.48–5.18; $P = 0.001$], hypertension



No of patients	0y	5y	10y	15y	20y
	4702	2960	1703	691	54

Age (years)	0y	5y	10y	15y	20y
<40	779	513	291	138	19
≥40- <50	973	622	387	193	20
≥50 -<60	1524	951	545	261	10
≥60	1426	874	480	99	5

Hypertension	0y	5y	10y	15y	20y
+	919	594	342	137	8
−	3783	2366	1361	554	46

	0y	5y	10y	15y	20y
Liver cirrhosis	494	330	197	78	5
Chronic hepatitis	4208	2630	1506	614	49

Efficacy	0y	5y	10y	15y	20y
HCV clearance	2125	1305	733	240	11
HCV non-clearance	2577	1655	970	451	43

Fig. 2. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke in total HCV patients treated with IFN therapy. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of age. **Panel C**: Cumulative development rate of ischemic stroke based on the difference of blood pressure. **Panel D**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of liver fibrosis. **Panel E**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of interferon efficacy.

TABLE II. Predictive Factors for the Development of Intracerebral Hemorrhagic Stroke

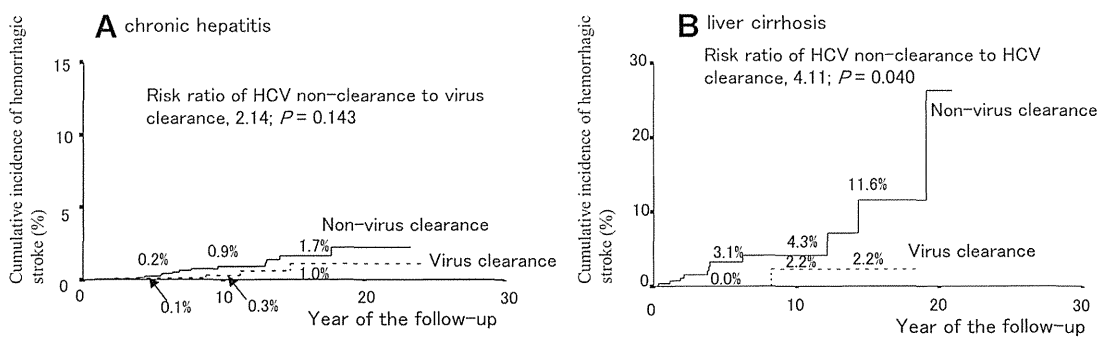
	Univariate analysis		Cox regression	
Variables	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years, per 10)	3.55 (1.96–6.43)	<0.001	2.77 (1.48–5.18)	0.001
Gender (M/F)	1.26 (0.65–2.44)	0.334		
BMI (≥22/<22)	0.97 (0.75–1.24)	0.767		
Diabetes (+/−)	3.40 (1.26–9.15)	0.015		
Hypertension (+/−)	4.07 (1.94–8.54)	<0.001	2.30 (1.09–4.83)	0.021
Smoking index (≥20/<20)[a]	2.12 (0.95–4.76)	0.068		
Total alcohol intake (kg, ≥200/<200)[a]	1.10 (0.53–4.37)	0.138		
AST (IU/L, ≥34/<34)	2.79 (1.17–6.66)	0.020		
ALT (IU/L, ≥36/<36)	2.68 (1.14–6.29)	0.023		
GGT (IU/L, ≥109/<109)	1.28 (0.610–1.89)	0.655		
Albumin (g/dl, <3.9/≥3.9)	2.96 (1.24–7.09)	0.015		
Triglyceride (mg/dl, ≥100/<100)	1.19 (0.83–1.49)	0.283		
Total cholesterol (mg/dl, <150/≥150)	1.06 (0.48–1.91)	0.936		
HDL-C (mg/dl, ≥40/<40)	0.96 (0.38–2.50)	0.960		
LDL-C (mg/dl, ≥120/<120)	0.81 (0.50–2.51)	0.572		
Platelet ($\times 10^4$/mm^3, <15/≥15)	3.22 (1.41–7.35)	0.005		
Histological diagnosis (cirrhosis/non-cirrhosis)	7.40 (3.30–16.77)	<0.001	4.50 (2.07–9.78)	<0.001
Combination of ribavirin (+/−)	0.80 (0.25–2.54)	0.701		
Type of IFN (α/β)	1.29 (0.65–2.33)	0.116		
Total dose of IFN (MU, ≥500/<500)	0.87 (0.39–1.99)	0.744		
HCV genotype (1/2)	1.53 (0.62–3.80)	0.360		
HCV RNA (log IU/ml, ≥5/<5)	1.35 (1.02–1.79)	0.035		
Efficacy (HCV: non-clearance/clearance)	2.98 (1.13–6.59)	0.020	3.22 (1.22–8.53)	0.018

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GGT, gamma-glutamyltransferase; HCV, hepatitis C virus; IFN, interferon.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.

(hazard ratio: 2.30; 95% CI 1.09–4.83; $P = 0.021$), liver cirrhosis (hazard ratio: 4.50; 95% 2.07–9.78; $P < 0.001$), and HCV non-clearance (hazard ratio: 3.22; 95% CI 1.22–8.53; $P = 0.018$). Figure 2B–E shows the cumulative incidence of hemorrhagic stroke based on difference of age, blood pressure, liver fibrosis, and efficacy of IFN therapy.

Hemorrhagic Stroke Based on the Difference of Liver Fibrosis and Efficacy

Figure 3A,B shows the cumulative incidence of intracerebral hemorrhagic stroke based on the difference of liver fibrosis and efficacy of IFN therapy. As shown in Figure 3B, HCV clearance reduced



Efficacy	0y	5y	10y	15y	20y
HCV clearance	1929	1180	627	217	9
HCV non-clearance	2235	1450	849	397	40

Efficacy	0y	5y	10y	15y	20y
HCV clearance	174	125	76	23	2
HCV non-clearance	311	205	121	55	3

Fig. 3. **Panel A**: Cumulative development rate of intracerebral hemorrhagic stroke based on difference of efficacy after interferon treatment in HCV patients with chronic hepatitis. **Panel B**: Cumulative development rate of intracerebral hemorrhagic stroke based on the difference of efficacy after interferon treatment in HCV patients with liver cirrhosis.

TABLE III. Comparison in Clinical Backgrounds Between HCV Clearance and HCV Non-Clearance in Patients With Liver Cirrhosis

	HCV clearance group	HCV non-clearance group	*P*-value
N	174	311	
Age (years)	56.7 ± 9.6	57.0 ± 9.9	0.721
Gender (M/F)	108/66	184/127	0.562
BMI	23.8 ± 3.7	23.6 ± 3.5	0.479
BP (systolic, mmHg)	132 ± 18	131 ± 17	0.791
BP (diastolic, mmHg)	80 ± 11	79 ± 12	0.775
Total alcohol intake (kg)[a]	112 ± 97	128 ± 101	0.057
Smoking index[a]	6.2 ± 10.7	5.9 ± 10.2	0.129
AST (IU/L)	33 ± 20	73 ± 47	<0.001
ALT (IU/L)	34 ± 28	79 ± 61	<0.001
GGT (IU/L)	24 ± 26	61 ± 65	<0.001
Albumin (g/dl)	3.7 ± 0.4	3.5 ± 0.4	0.149
Triglyceride (mg/dl)	110 ± 47	104 ± 45	0.243
Cholesterol (mg/dl)	157 ± 29	161 ± 31	0.373
HDL-C (mg/dl)	42 ± 12	45 ± 12	0.257
LDL-C (mg/dl)	96 ± 26	95 ± 30	0.748
Fasting plasma glucose (mg/dl)	104 ± 22	109 ± 26	0.085
HbA_{1C} (%)	5.7 ± 1.2	6.0 ± 1.3	0.024
Platelet ($\times 10^4/mm^3$)	14.1 ± 6.2	17.3 ± 5.4	0.097
HCV genotype (1b/2a/2b/other)[b]	75/72/24/3	209/54/15/33	<0.001
HCV RNA (log IU/ml)[b]	5.32 ± 1.12	6.38 ± 1.00	<0.001
IFN monotherapy/combination therapy[c]	110/64	232/79	0.012

Data are number of patients or mean ± standard deviation, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyltransferase; HbA_{1C}, hemoglobin A_{1C}; HCV, hepatitis C virus; HDL, high density lipoprotein; IFN, interferon.
[a]Smoking index is defined as package per day × year; total alcohol intake and smoking index indicate the sum before and after first consultation.
[b]Value before IFN treatment.
[c]Outbreak of IFN monotherapy: natural IFN alpha, 252 cases; natural IFN beta, 90 cases; total dose of IFN = 518 ± 156 MU.
Outbreak of combination therapy: natural IFN beta + ribavirin, 41 cases, total dose of IFN = 490 ± 171 MU, total dose of ribavirin = 151 ± 64 g; peg IFN alpha 2b + ribavirin, 102 cases, total dose of peg IFN = 3.96 ± 1.03 mg, total dose of ribavirin = 188 ± 51 g.

hemorrhagic stroke to one-fourth in cirrhotic patients. Table III shows the clinical backgrounds between HCV clearance and HCV non-clearance in patients with liver cirrhosis. There are significant differences in AST, ALT, GGT, HCV genotype, HCV RNA, and HbA_{1C} between HCV clearance group and HCV non-clearance group. However, there are no significant differences in age and hypertension between HCV clearance group and HCV non-clearance group.

DISCUSSION

The incidence of hemorrhagic stroke after the termination of IFN therapy in HCV patients has been described in the present study. The strengths of the present study are a prolonged follow-up in the large numbers of patients included.

The present study shows several findings with regard to the cumulative incidence and predictive factors for hemorrhagic stroke after IFN therapy for HCV patients. First, intracranial hemorrhagic stroke occurred significantly when patients had advanced age of ≥60 years, hypertension, liver cirrhosis, and HCV non-clearance. Several authors have reported that the most common risk factor for hemorrhagic stroke is aging, high levels of blood pressure [Turin et al., 2010; O'Donnell et al., 2010; Naidech, 2011; Cervera et al., 2012]. In addition, antiplatelet and anticoagulant medications also increase the risk of hemorrhagic stroke [Cervera et al., 2012]. Our results evaluated hemorrhagic stroke in HCV patients agreed with these reports concerning aging and hypertension.

Second, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients. In general, patients with advanced liver fibrosis have often the hemorrhagic tendency due to prothrombin deficit and platelets diminution. Thus, our result suggests that the HCV clearance prevent the aggravation of prothrombin deficit and platelets diminution. Our previous reports have indicated that HCV clearance reduces type 2 diabetes mellitus [Arase et al., 2009], bone fracture [Arase et al., 2010], and chronic kidney disease [Arase et al., 2011]. In the present study, HCV clearance reduced the incidence of intracerebral hemorrhagic stroke. In particular, HCV clearance reduced intracerebral hemorrhagic stroke to about one-fourth in cirrhotic patients.

A hemorrhagic stroke is the rapid loss of brain function due to hemorrhage. As a result, a hemorrhagic stroke is a medical emergency and can cause permanent neurological damage and death. Recently, the life span has been long in Japan. Thus, in near the future, a large number of patients with HCV will be >60 years of age. A hemorrhagic stroke might be increasing in HCV positive patients in aging society. Our results show that physicians in charge of HCV

patients with hypertension, liver cirrhosis, and HCV non-clearance should be noted the development of hemorrhagic stroke.

The present study was limited by a retrospective cohort trial. Another limitation of the study was that patients were treated with different types of antivirus therapy for different duration. In addition, these patients were treated with different types of drugs for diabetes, hypertension, and dyslipidemia during follow-up. Finally, our cohort contains Japanese subjects only. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

In conclusion, HCV clearance reduced hemorrhagic stroke to about one-fourth in cirrhotic patients.

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Prevention of Disease Progression with Anti-Inflammatory Therapy in Patients with HCV-Related Cirrhosis: A Markov Model

Kenji Ikeda[a, b] Yusuke Kawamura[a, b] Masahiro Kobayashi[a, b] Taito Fukushima[a, b] Hitomi Sezaki[a, b] Tetsuya Hosaka[a, b] Norio Akuta[a, b] Satoshi Saitoh[a, b] Fumitaka Suzuki[a, b] Yoshiyuki Suzuki[a, b] Yasuji Arase[a, b] Hiromitsu Kumada[a, b]

[a]Department of Hepatology, Toranomon Hospital, and [b]Okinaka Memorial Institute for Medical Research, Tokyo, Japan

Key Words

Hepatitis C · Hepatocellular carcinoma · Interferon · Glycyrrhizin · Carcinogenesis · Markov model · Anti-inflammatory therapy

Abstract

Background: The significance of anti-inflammatory therapy has not been fully evaluated in hepatitis C virus (HCV)-related cirrhosis. ***Patients and Methods:*** We analyzed stepwise progression rates from cirrhosis to hepatocellular carcinoma (HCC) and to death using a Markov model in 1,280 patients with HCV-related cirrhosis. During the observation period, 303 patients received interferon and 736 received glycyrrhizin injections as anti-inflammatory therapy. ***Results:*** In the entire group, annual progression rates from cirrhosis to HCC and from cirrhosis to death were 6.8 and 1.9%, and the rate from HCC to death was 19.0%. When sustained virological response (SVR) or biochemical response (BR) was attained with interferon, the annual rate to HCC decreased to 2.6%. On the contrary, the progression rates to HCC and to death in the patients without SVR and BR were 7.2 and 2.0%, respectively ($p < 0.0001$). Continuous interferon administration significantly decreased the carcinogenesis rate to 5.5% ($p = 0.0087$). In the analysis of the remaining patients with high alanine transaminase of 75 IU/l or more but without interferon response or without interferon administration, glycyrrhizin injection significantly decreased annual non-progression probability (no glycyrrhizin 88.0% vs. glycyrrhizin therapy 92.3%, $p = 0.00055$). ***Conclusion:*** Glycyrrhizin injection therapy is useful in the prevention of disease progression in interferon-resistant or intolerant patients with HCV-related cirrhosis.

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Introduction

Hepatitis C virus (HCV) is one of the principal etiologies of hepatocellular carcinoma (HCC), with high morbidity and mortality rates in many countries [1–5]. Because interferon has anti-viral, anti-fibrotic and anti-inflammatory properties, it is still a main agent in the treatment of chronic hepatitis C [6, 7]. Many authors have described interferon as capable of preventing hepatocarcinogenesis and prolonging patient survival [8–13]. The radical eradication of HCV by interferon greatly depends on viral load, HCV subtype, certain mutations of the hepatitis virus gene, liver histology, mode of interferon administration and various host factors, including

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Kenji Ikeda, MD, PhD
Department of Hepatology, Toranomon Hospital
Toranomon 2-2-2, Minato-ku
Tokyo 105-8470 (Japan)
E-Mail ikedakenji@tora.email.ne.jp

the patient's age [10, 13, 14]. When a significant side effect occurs during interferon therapy, cessation or early withdrawal of the therapy often leads to an unsuccessful result. Early withdrawal and treatment failure is usually more common in patients with an advanced stage of liver disease.

Adverse effects of interferon are more commonly found in patients with cirrhosis, and hematological disorders often necessitate cessation of interferon before the therapy is complete. As a result, interferon is considered less effective in the advanced stage of hepatitis. Liver cirrhosis is usually associated with patients aged 55–60 years or older; the adverse effects of interferon-based anti-viral therapy are prevalent in this age group, resulting in low overall compliance for long-term therapy. Because the severity of chronic liver disease is closely associated with the response to interferon therapy [14–16], the sustained response rate is often low in patients with cirrhosis. Furthermore, an older patient with cirrhosis has a very high risk of carcinogenesis and mortality because fibrotic stage is correlated with a patient's age. The role of interferon in suppression of the carcinogenesis rate is therefore likely to be less significant in patients with cirrhosis en masse. There have been several clinical attempts to administer interferon for HCV-related cirrhosis to suppress the hepatocellular carcinogenesis rate [8, 9, 11, 12, 17–19]. However, there have been conflicting reports about the therapeutic value of interferon for this purpose. Some studies have shown a beneficial effect of interferon in reducing carcinogenesis [8, 9, 12, 18], but other reports have not [11, 17, 19].

When interferon fails to eliminate HCV RNA in a patient, long-term administration of interferon often shows anti-carcinogenic action through stabilization of alanine transaminase (ALT) and suppression of the necro-inflammation of hepatocytes [20]. For patients who do not respond to long-term interferon therapy, as shown by persistently high ALT values, glycyrrhizin injection therapy is available in several countries, including some countries in Asia and Europe. A glycyrrhizin-containing product, Stronger Neo-Minophagen C™ (SNMC; Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan), is widely used in Japan for suppression of hepatitis activity and for prevention of disease progression in patients with hepatitis B virus- and HCV-induced chronic hepatitis. Glycyrrhizin has been reported to mitigate hepatic inflammation by suppressing elevated ALT levels and preventing disease progression [21–24]. We previously reported the favorable effects of long-term administration of glycyrrhizin against hepatocellular carcinogenesis in patients with interferon-naïve and interferon-resistant chronic hepatitis C [25, 26].

In order to elucidate whether long-term glycyrrhizin injection therapy suppresses hepatocarcinogenesis and mortality rates in patients with interferon-resistant cirrhosis, we retrospectively analyzed a large cohort of patients with HCV-related cirrhosis in a single institution. The principal aims of our study were to show the clinical role of glycyrrhizin in advanced liver disease, and to determine whether glycyrrhizin can be used as an anti-inflammatory therapy.

Patients and Methods

Study Population and Analyzed Cohorts

A total of 1,358 consecutive patients with hepatitis C were diagnosed as having liver cirrhosis at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan, from 1974 to 2007. They had positive anti-HCV antibody, detectable HCV RNA (nested PCR), and negative hepatitis B surface antigen. Anti-HCV and HCV RNA were assayed using stored frozen sera. Among the 1,358 consecutive patients with hepatitis C, 78 patients were excluded from the study based on meeting one or more of the following exclusion criteria: (1) possible association with HCC; (2) association with hemochromatosis, autoimmune liver disease, primary biliary cirrhosis, α1-antitrypsin deficiency or Wilson disease; (3) daily alcohol ingestion of 75 g or more; (4) α-fetoprotein of 400 ng/ml or higher; (5) a short follow-up period of 6 months or less, or (6) Child-Pugh stage C liver disease because of the substantial difference in carcinogenesis in these patients [27–29].

The remaining 1,280 patients with HCV-positive liver cirrhosis were retrospectively analyzed for hepatocellular carcinogenesis and mortality. Among them, 754 patients (59.4%) were diagnosed as having cirrhosis by histopathological findings with peritoneoscopy and biopsy, and the remaining 526 (40.6%) were diagnosed with clinical findings: rough-surfaced liver on imaging (ultrasonography or computerized tomography, CT), plus endoscopic finding of esophageal varices, overt ascites or indocyanine green retention rate at 15 min of 30% or more. There were 744 men and 536 women, with a median age of 59 years (range 22–86). They were observed for a median of 8.1 years (table 1). A total of 231 patients (18.0%) were lost to follow-up during the observation period.

Interferon Treatment and Evaluation of Effects

Among the 1,280 patients with cirrhosis, 303 patients (23.7%) received interferon therapy with or without ribavirin. Among the 303 patients receiving interferon therapy, 252 received interferon-α and 51 received interferon-β therapy. For dosages, 258 patients received at least 6 million IU/day, and the other 45 patients received no more than 3 million IU/day as their initial anti-viral therapy. Of 303 patients receiving interferon, 52 patients received interferon daily for the first 2–8 weeks and then 2–3 times per week for the following 24–72 weeks. The other 251 patients received interferon 3 times per week for 24–72 weeks. The median administration period was 26.0 weeks (range 4–548).

Table 1. Clinical features of the study group: 1,280 patients with liver cirrhosis caused by hepatitis C

Demography	
Male	744 (58.1)
Female	536 (41.9)
Age, years	59 (22–86)
Decompensated cirrhosis	134 (10.5)
History of blood transfusion	549 (42.9)
Total alcohol intake >500 kg	200 (15.6)
Presence of diabetes mellitus	249 (19.5)
Observation period, years	8.1 (0.5–30.6)
Laboratory data	
ICG R15, %	27 (2–96)
Bilirubin, mg/dl	1.0 (0.2–7.7)
Albumin, g/dl	3.7 (1.6–5.1)
Aspartate transaminase, IU/l	66 (14–1313)
ALT, IU/l	62 (4–570)
Platelet count, $\times 10^3/mm^3$	104 (20–398)
Prothrombin time, %	82 (11–117)
Hepatitis C subtype	
1	821 (75.7)
2	254 (23.4)
Other	9 (0.8)
Treatment after diagnosis of cirrhosis	
Interferon with/without ribavirin	303
Glycyrrhizin injection	736
Ursodeoxycholic acid	615

Data are presented as the median value with range in parentheses, or n with percentages in parentheses. ICG R15 = Indocyanine green retention rate at 15 min.

Almost all patients who received interferon therapy showed varying degrees of influenza-like symptoms, leukocytopenia and thrombocytopenia. Eight patients discontinued interferon therapy because of significant adverse reactions: depression in 2 patients, severe cytopenias in 2, marked anorexia in 1, malaise in 2 and retinopathy in 1 patient. No patients developed decompensated liver disease with ascites, encephalopathy, jaundice or variceal bleeding.

The effects of interferon therapy were classified according to the elimination of HCV RNA and the levels of ALT for 6 months after the end of the treatment. Sustained virological response (SVR) was defined as persistent disappearance of HCV RNA after therapy. Biochemical response (BR) was defined as normal ALT values without elimination of HCV RNA for at least 6 months after therapy. No response (NR) was defined as persistently abnormal or only transient normalization of ALT for a period of less than 6 months. Because 73 patients (24.1%) were still undergoing their course of interferon therapy, the evaluation was conducted in 230 (75.9%) of the 303 patients.

Glycyrrhizin Injection (SNMC) Therapy

Glycyrrhizin therapy was performed using intravenous injections of SNMC™ (Minophagen Pharmaceutical Co. Ltd.). The preparation contains 0.2% (40 mg) glycyrrhizinic acid as the main active constituent, 2% (400 mg) glycine and 0.1% (20 mg) L-cysteine in a 20-ml ampule.

Of 376 chronic hepatitis patients with interferon resistance or who did not receive interferon injection therapy, 264 patients underwent glycyrrhizin injection therapy and the remaining 112 patients did not receive therapy until the end of observation. The purpose of glycyrrhizin injection therapy was to suppress elevated ALT levels and to prevent disease progression in all the patients. In patients for whom the treatment was regarded as effective with respect to ALT levels, the treatment was usually continued for as long a period as possible. As a result, a daily dose of 100 ml of SNMC was administered three times a week for a median period of 4.9 years (range 0.1–24.1) in the glycyrrhizin-treated group.

Certain patients with a high ALT value did not receive glycyrrhizin injection for a variety of reasons. These included the refusal of intravenous treatment, a difficulty in frequently visiting the clinic for the injection, inappropriate superficial veins for repeated injection, negativism towards the handling of intravenous therapy by the doctors in charge, and so on. Those patients who did not receive glycyrrhizin injection therapy in spite of a high ALT often received pills of ursodeoxycholic acid as an anti-inflammatory therapy.

Follow-Up of Patients and Diagnosis of HCC

Follow-up of the patients was made on a monthly to tri-monthly basis after the initial visit. Imaging diagnosis was made one or more times per year with ultrasonography, CT or magnetic resonance imaging. HCC was diagnosed by its typical hypervascular characteristics on CT, magnetic resonance imaging or angiography. When combined use of imaging modes could not demonstrate a typical image of HCC, a fine-needle biopsy was obtained for microscopic examination. The imaging diagnosis was similarly performed among those patients with interferon therapy, glycyrrhizin therapy and without therapy.

Statistical Analysis and Markov Model

Standard statistical measures and procedures were used. The χ^2 test, Fisher exact test and Mann-Whitney U test were used to analyze the differences in demography and laboratory findings. Progression and survival rates were analyzed using the Kaplan-Meier technique [30] with the log-rank test. A Markov model [31] was used to analyze the transition rates from liver cirrhosis to appearance of HCC, and to death. A homogenous Markov chain consisted of three states (fig. 1). These were liver cirrhosis, appearance of HCC and death as an absorbing state from where no transitions to the other states occurred. The model was based on the following principles: (1) the three states are mutually exclusive and collectively exhaustive; (2) the Markov assumption is that the current state has no memories of prior states; (3) the time intervals are uniform, and (4) the transition probabilities are constant and time independent. The first and second items here define a Markov chain, whereas the third and fourth items characterize a homogenous Markov chain [32]. Patient data were regarded as censored at the time of the last date of observation, in the evaluation of survival analysis and Markov analysis.

A p value <0.05 in the two-tailed test was considered significant. Data analysis was performed using IBM SPSS Statistics version 18 [33]. The Human Ethics Review Committee of Toranomon Hospital approved the study protocol.

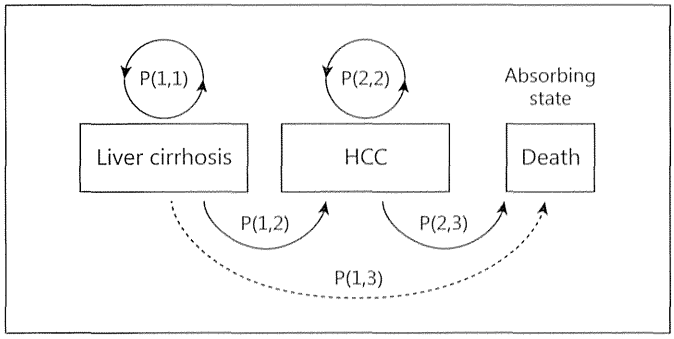

Fig. 1. Markov state transition diagram of liver cirrhosis. Three states were defined: liver cirrhosis without development of HCC, liver cirrhosis-associated HCC, and death. Of these, death was the absorbing state from which no transitions to the other states occurred. The transition in one cycle (1 year) is shown. Arrows connecting two different states indicate observed transitions. The figure represents a probability diagram of the entire study group. All patients were initially at the stage of liver cirrhosis, but transitions to HCC stages gradually increased with time.

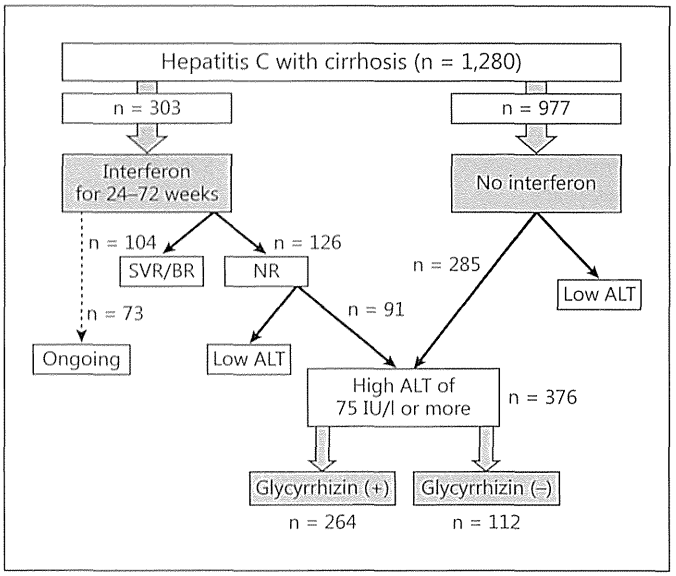

Fig. 2. Clinical courses of patients with cirrhosis. Among 303 patients who received interferon therapy, there were 104 patients who had SVR or BR, and 126 patients who had NR. The remaining 73 patients continued to receive long-term interferon therapy. Among 376 patients with a high ALT value of 75 IU/l or more, with or without a history of interferon therapy, 264 patients underwent glycyrrhizin injection therapy and 112 did not receive glycyrrhizin.

Results

Effects of Interferon and Anti-Inflammatory Treatment

Among the 303 patients who underwent interferon therapy with or without ribavirin, 79 patients (26.1%) showed HCV RNA clearance (SVR effect), and 25 patients (8.3%) showed a BR with normal ALT values for 6 months or longer. One hundred and twenty-six patients (41.6%) showed NR after cessation of interferon. The remaining 73 patients (24.1%) continued intermittent interferon administration for 1 year or longer.

Among the 977 patients who did not receive interferon therapy, plus the 126 patients who received interferon with NR, a high ALT value of 75 IU/l or more was found in 376. Of these patients, 264 (70.2%) underwent long-term glycyrrhizin injection therapy and the other 112 (29.8%) did not receive glycyrrhizin (fig. 2).

Crude Hepatocellular Carcinogenesis and Survival Rates in the Entire Study Group

Cumulative hepatocellular carcinogenesis rates were calculated in all 1,280 study patients with HCV-related cirrhosis. The carcinogenesis rates were 16.4, 29.2, 37.3, 51.6, 65.0 and 69.5% at the end of the third, fifth, seventh, tenth, fifteenth and twentieth years, respectively (fig. 3a). The cumulative survival rates were 93.0, 86.3, 77.1, 61.9, 39.3 and 25.4% at the same time points (fig. 3b).

Probabilities for Transition among the Three Disease States according to the Results of Interferon and Anti-Inflammatory Treatment

In the matrix of the entire study group, 6.8% (562/8,273) of the patients with liver cirrhosis progressed to HCC annually, and 1.9% (157/8,273) died. The remaining 91.3% (7,554/8,273) of the patients remained in the stage of liver cirrhosis after 1 year. Similarly, 19.0% (423/2,228) of the patients in the stage of HCC died, and 81.0% (1,805/2,228) of the patients remained in the stage of HCC annually (table 2).

The results are shown in table 3 as a matrix of transition probabilities for three subsets composed of treatments (SVR or BR, NR or no interferon, and continual interferon) stratified by three states (cirrhosis, HCC, and death). The probabilities for transition from liver cirrhosis to HCC and from liver cirrhosis to death were significantly lower in patients who achieved SVR or BR [2.6% (20/778) and 0.6% (5/778)] than in patients with NR or no interferon therapy [7.2% (542/7,494) and 2.0% (151/7,494); $\chi^2 = 32.4$, $p < 0.0001$]. The probabilities for transition from liver cirrhosis to HCC and from liver cirrhosis to death were significantly lower in patients who