Single-nucleotide polymorphism genotyping

We genotyped each patient for two single-nucleotide polymorphism (SNP)s: rs12979860, an IL28B (interleukin 28B) SNP previously reported to be associated with therapeutic outcome [17], and rs1127354, an inosine triphosphatase (ITPA) SNP reported to be associated with ribavirin-induced anaemia [18]. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip (Illumina, San Diego, CA, USA) or the Invader or TaqMan assay, as described previously [19,20].

Drug concentrations

Blood samples were collected immediately prior to the administration of TVR or RBV in the morning on days 8, 15 and 29. Serum concentrations of TVR were determined at day 8 (week 1) using a high-performance liquid chromatographic (HPLC) apparatus fitted with a mass spectrometer (LC-MS/MS) (Mitsubishi Chemical Medience Corporation, Tokyo, Japan), and the serum concentration of RBV was measured at day 8 (week 1), day 15 (week 2) and day 29 (week 4) by HPLC (SRL, Inc. Tokyo, Japan) using serum stored at −80 °C.

Renal function

To assess the renal function, we used the estimated glomerular filtration rate (eGFR), which was calculated according to the equation [$194 \times (\mathrm{Scr}^{-1.094}) \times (\mathrm{age}^{-0.287}) \times$ (0.739 for women) (mL/min/1.73 m^2) serum creatinine (Scr)], as established by the Japanese Society of Nephrology in 2008 [21].

Statistical analysis

Continuous variables between groups were compared using the Mann–Whitney *U*-test, and categorical variables were compared using the Fisher's exact test. The correlation between the two groups was calculated using Spearman's rank correlation coefficient. Statistical analyses were performed using the statistical software SAS version 9.1 (SAS Institute Inc., Cary, NC, USA); a P value of <0.05 was considered significant.

RESULTS

Telaprevir dose and patient background

The TVR dose was administered according to initial Hb level and gender. The dose per weight of TVR and RBV was significantly higher in the patients receiving the 2250-mg TVR dose; however, the dose per weight of PEG did not differ according to the TVR dose. With regard to background factors, an increased age, higher proportion of women, decreased height and weight, lower white blood cell count and lower levels of Hb and eGFR were noted in the 1500-mg TVR dose group (Table 1).

Telaprevir serum concentration

The TVR serum concentration (trough value) at day 8 (week 1) of triple therapy was measured in 65 of 68 cases. The TVR concentration varied widely (1076–4598 ng/mL). The mean TVR serum concentration of patients receiving the 2250-mg TVR dose was higher than that of patients receiving the 1500-mg TVR dose (2739 ± 833 ng/mL *vs* 2361 ± 838 ng/mL, respectively); however, the difference was not statistically significant ($P = 0.075$). The patients' background factors were used as independent variables in the multiple regression analysis (Table 1), and the TVR serum concentration was estimated using the following formula: TVR concentration (ng/mL) = 56.2 × TVR (mg/kg)− 37.6 × height (cm) + 6878.4 ($R'^2 = 0.3500$, $P < 0.001$) (Fig. 1). In addition, the dose per weight of TVR (mg/kg) and the TVR serum concentration were significantly correlated ($r = 0.4795$, $P < 0.001$) (Fig. 2).

Correlation of telaprevir serum concentration, dose per weight of telaprevir (mg/kg) and decline in renal function

The patients' eGFR declined at an initial stage during triple therapy, and the decline continued for the duration of TVR treatment [baseline: 85.8, week 1: 69.6, week 2: 70.2, week 4: 69.2, week 8: 66.6, week 12: 72.5 (mL/min/1.73 m^2)] (Figure S1). The total TVR serum concentration (trough value) at week 1 of the triple therapy was not correlated with the delta eGFR; however, the dose per body weight of TVR (mg/kg) was significantly correlated with delta eGFR at week 1 and week 4, but not at week 8 (Fig. 3).

Correlation of renal function and ribavirin serum concentration

There was no correlation between the dose per weight of RBV (mg/kg) and eGFR or delta eGFR at week 1 ($P = 0.6422$ and $P = 0.1152$, respectively). However, at week 1, there was a significant negative correlation between eGFR and RBV serum concentration, and a significant positive correlation between delta eGFR and RBV serum concentration ($r = -0.3694$, $P = 0.0025$; $r = 0.3189$, $P = 0.0096$, respectively) (Fig. 4). By multiple regression analysis, the serum concentration of RBV at week 1 was estimated using the formula: RBV concentration (ng/mL) = 413.5 × sex (male = 1, female = 2) + 12.8 × age (years) + 163.5 × RBV (mg/kg)−5.9 × eGFR (1 week)−1291.6 ($R'^2 = 0.4631$, $P < 0.001$) (Fig. 1). Thus, in addition to age, sex and the dose per weight of

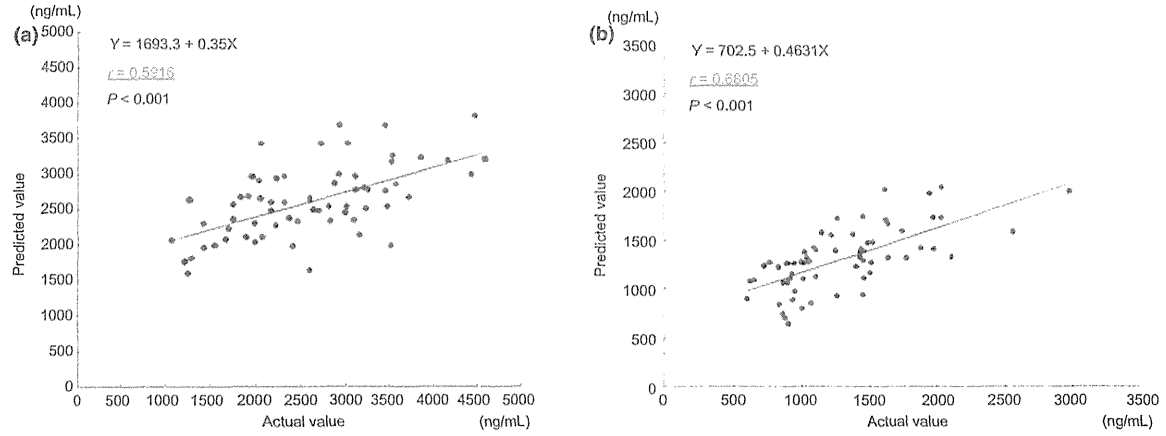

Fig. 1 (a) Correlation of TVR serum concentration actual value and predicted value, and (b) correlation of RBV serum concentration actual value and predicted value.

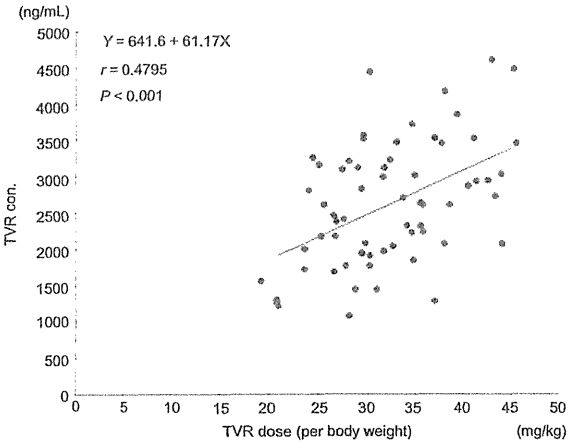

Fig. 2 Correlation of TVR serum concentration and quantity of TVR (mg/kg)

RBV (mg/kg), eGFR was also correlated with the RBV serum concentration.

Correlation of telaprevir, ribavirin serum concentration and haemoglobin level

The TVR serum concentration at week 1 was significantly negatively correlated with Hb levels at week 3, 6, 7 and 8 of treatment and was significantly positively correlated with the delta Hb level only at week 3 of treatment. The RBV serum concentration at week 1 showed a significant negative correlation with the Hb levels from week 2 to week 8 and a significant positive correlation with the delta Hb levels from week 1 to week 8. Moreover, the RBV serum concentration at week 2 and week 4 showed a significant negative and positive correlation with the Hb levels and the delta Hb levels, respectively (Table S1).

DISCUSSION

As RBV is usually eliminated by renal filtration, the development of renal failure would result in the accumulation of RBV, particularly in red blood cells, and may induce haemolytic anaemia. Triple therapy that includes TVR has two well-known serious side effects: anaemia and dermatopathy. In addition to these side effects, a decline in renal function, which was noted in Japan after TVR was made commercially available, is now recognized as a significant problem; this decline in renal function was not noted with PEG-IFN and RBV combination therapy. Renal dysfunction has also recently been associated with boceprevir use [22,23]. The mechanism responsible for the decline in renal function caused by TVR remains unknown; however, the risk factors in Japanese patients include receiving a 2250-mg TVR dose, advanced age and low Hb levels at the start of the therapy.

The serum concentration of TVR reportedly reaches a steady state in 2–7 days of treatment [24,25]. In the present study, the trough serum concentration of TVR at day 8 of triple therapy was estimated through multiple regression analysis using the formula: TVR (ng/mL) = 56.2 × TVR (mg/kg)−37.6 × height (cm) + 6878.4. The dose per weight of TVR (mg/kg) was the strongest determinant of the TVR serum concentration. The renal function impairment in patients treated with the TVR/PEG-IFN/RBV combination therapy was noted at an early stage in the treatment and persisted throughout the TVR treatment duration, but gradually improved after TVR treatment was discontinued. In patients receiving the TVR/PEG-IFN/RBV combination therapy, although the serum TVR concentration at week 1 was not significantly correlated with the decline in renal function during week 1, the dose per weight of TVR (mg/kg) showed a significant correlation with the decline in renal function during week 1. Thus,

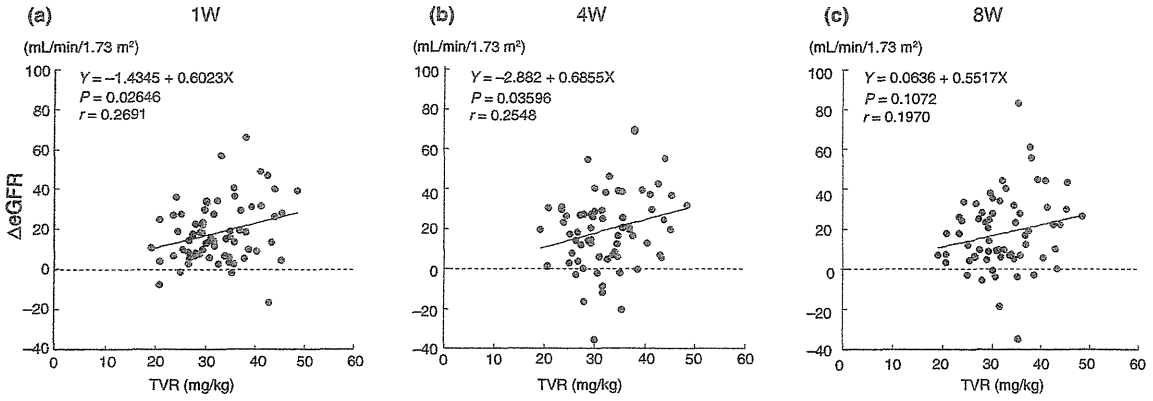

Fig. 3 Correlation between TVR (mg/kg) and delta eGFR.

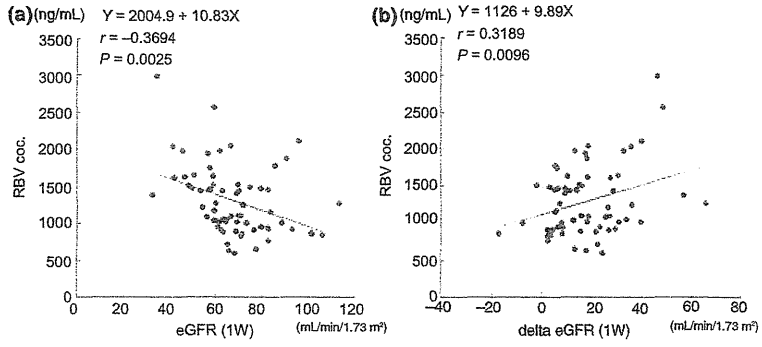

Fig. 4 Correlation between RBV concentration (1W) and eGFR (1W) and delta eGFR (1W)

the degree of renal function impairment worsened with an increase in the dose per weight of TVR (mg/kg). Furthermore, because RBV is eliminated by renal filtration [15], it is expected that a decline in renal function will result in an increase in serum RBV concentration. In the present study, the serum concentration of RBV at week 1 showed a significant negative correlation with eGFR and significant positive correlation with delta eGFR. The RBV serum concentration was estimated through multiple regression analysis using the formula: RBV concentration (ng/mL) = 413.5 × sex (male = 1, female = 2) + 12.8 × age (years) + 163.5 × RBV (mg/kg) − 5.9 × eGFR (week 1) − 1291.6. In addition to sex, age and dose per weight of RBV, the eGFR at week 1 was a regulating factor for RBV serum concentration. After examining the relationships among TVR serum concentration, RBV serum concentration, and Hb level, the TVR serum concentration at week 1 was negatively correlated with the Hb level at week 3, 6, 7 and 8 and was positively correlated with the delta Hb level at week 3. The RBV serum concentration was negatively correlated with the Hb level from week 2 to 8 and positively correlated with the delta Hb level from week 1 to 8. Similarly, the serum RBV concentration at weeks 2 and 4 was correlated with the Hb level and the delta Hb level, respectively.

In patients receiving TVR-containing triple therapy, the development of anaemia can lead to dose reduction or discontinuation of the drug. The administration of TVR or RBV alone can cause anaemia. However, the renal dysfunction caused by TVR can exacerbate this problem by increasing the serum concentration of RBV and thus increase the severity of haemolytic anaemia.

Because TVR was administered at a fixed dose of 2250 mg/day during the third phase of the clinical trial for TVR/PEG-IFN/RBV combination therapy in Japan, the dose per weight of TVR varied widely (25.7–55.3 mg/kg) [13]. Moreover, by multivariate analysis, we noted that the factor that contributed significantly to a decline in the Hb level to less than 8.5 g/dL – which necessitated the discontinuation of TVR/PEG-IFN/RBV combination therapy – was a relatively high dose per weight of TVR [26]. In the present study, although the TVR dose was reduced to 1500 mg/day in a few female patients and in those with low baseline Hb level, the dose per weight of TVR varied widely (19.2–48.5 mg/kg). A decline in the eGFR at week 1 was related to the dose per weight of TVR, and the RBV serum concentration at week 1 was related to the decline in the eGFR at week 1; moreover, a higher RBV concentration at week 1 was related to a greater decline in Hb levels.

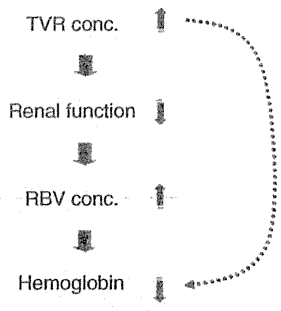

Fig. 5 Cascade reaction of RBV induced anaemia during triple therapy.

In summary, we noted a certain sequence of events that can occur during anti-HCV treatment that includes TVR: (i) the starting dose of TVR, when not calculated on a per weight basis, may be high, (ii) TVR causes a reduction in renal function, with a reduction in the excretion of RBV, (iii) the blood concentration of RBV rises and (iv) the increased RBV concentration, in addition to the inherent tendency for TVR to lower Hb levels, leads to haemolytic anaemia (Fig. 5). The declining Hb levels may require discontinuation of the regimen or reduction in the anti-HCV drug doses and, consequently, may result in a reduced antiviral effect. For patients requiring TVR/PEG-IFN/RBV combination therapy, the interactions of TVR and RBV should be considered while determining the optimal doses of TVR and RBV.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Correlation between serum concentration of RBV or TVR and Hb, as well as delta Hb levels.

Figure S1. The reduction in the eGFR is evident as early as week 1 of therapy and persists for the duration of treatment.

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Article

Endoscopic Color Doppler Ultrasonographic Evaluation of Gastric Varices Secondary to Left-Sided Portal Hypertension

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Abstract: Gastric varices that arise secondary to the splenic vein occlusion can result in gastrointestinal hemorrhaging. Endoscopic color Doppler ultrasonography (ECDUS) was performed in 16 patients with gastric varices secondary to splenic vein occlusion. This study retrospectively evaluated the role of ECDUS in the diagnosis of gastric varices secondary to splenic vein occlusion. Thirteen patients had co-existing pancreatic diseases: 8 with chronic pancreatitis, 4 with cancer of the pancreatic body or tail and 1 with severe acute pancreatitis. Of the remaining 3 patients, 1 had myeloproliferative disease, 1 had advanced gastric cancer, and the third had splenic vein occlusion due to an obscure cause. The endoscopic findings of gastric varices were: variceal form (F) classified as enlarged tortuous (F2) in 12 cases and large, coil-shaped (F3) in 4 cases, and positive for erosion or red color sign of the variceal surface in 4 cases and negative in 12 cases. ECDUS color flow images of gastric variceal flow clearly depicted a round fundal region at the center, with varices expanding to the curvatura ventriculi major of the gastric body in all 16 cases. The velocities of F3 type gastric varices were significantly higher than those of the F2 type. The wall thickness of varices positive for erosion or red color sign was significantly less than the negative cases. I conclude that ECDUS color flow images of gastric variceal flow depicted specific findings of gastric varices secondary to splenic vein occlusion at the round fundal region at the center, with varices expanding to the curvatura ventriculi major of the gastric body.

Keywords: endoscopic ultrasonography; color Doppler; gastric varices; left-sided portal hypertension; splenic vein occlusion

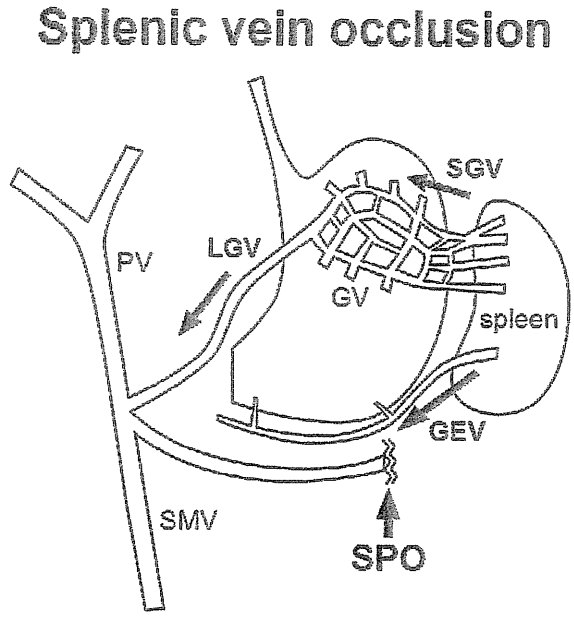
1. Introduction

Gastric variceal hemorrhage is a common complication of portal hypertension and is associated with higher rates of morbidity and mortality than hemorrhage of esophageal varices [1]. Although hemodynamic studies of gastric varices have been made worldwide [2–4], splenic vein occlusion is often clinically silent, presenting no obvious symptoms. However, gastric varices secondary to splenic vein occlusion can cause gastrointestinal hemorrhaging (left-sided portal hypertension) [5–8].

Splenic vein occlusion results in left-sided portal hypertension (characterized by gastric varices, splenomegaly and normal liver function) [9–11] that is secondary to various diseases [12–14]. The majority of splenic vein occlusions are the result of pancreatic diseases, including acute and chronic pancreatitis and pancreatic tumors.

Occlusion of the splenic vein causes the splenic venous flow to drain into collateral veins (the short gastric vein and left gastroepiploic vein) and this increased blood flow dilates the submucosal veins of the stomach, resulting in gastric varices (Figure 1). Because blood drainage is diverted by the coronary vein into the patent portal system, the presence of gastric varices without esophageal varices is a very specific sign of splenic vein occlusion [15].

Figure 1. Hemodynamics of gastric varices secondary to splenic vein occlusion (reproduction from Sato *et al.* [16].



GV: gastric varices PV: portal vein
SMV: superior mesenteric vein
SGV: short gastric vein LGV: left gastric vein
GEV: gastroepiploic vein
SPO: splenic vein occlusion

Although splenic vein occlusion is commonly silent clinically, this condition may cause gastrointestinal bleeding from gastric varices. In previous studies of splenic vein occlusion, gastrointestinal bleeding was reported to occur in 16%–78% of patients [8,9,13,17–20] Sutton *et al.* [9] found 53 cases of such occlusion, and reported a 64% incidence of upper gastrointestinal bleeding. Itzchak and Glickman [20] noted gastrointestinal hemorrhaging in only 3 of 19 patients with splenic vein occlusion. Sarin *et al.* [18] studied the prevalence of gastric varices in 568 patients with portal hypertension and reported that 7 of 9 patients (78%) with gastric varices due to splenic vein occlusion had a history of previous variceal bleeding. With recent improvements in cross-sectional imaging, Heider *et al.* [21] reported gastric variceal bleeding from pancreatitis-induced splenic vein thrombosis (with minimal symptoms) in only 4% of patients. Recently, Butler *et al.* [22] reported that the rate of pancreatitis-induced splenic vein thrombosis associated gastrointestinal bleeding is 12.3%.

The aim of this study was to investigate the role of ECDUS in the diagnosis of gastric varices secondary to splenic vein occlusion.

2. Subjects and Methods

2.1. Patients

Between January 1996 and December 2013, 205 patients with gastric varices that were found consecutively by routine upper endoscopy were retrospectively evaluated with ECDUS at Sapporo Kosei Hospital. This study group consisted of 16 patients with gastric varices due to splenic vein occlusion (13 males, 3 females, ages 33 to 79 years (mean: 60.5 years)) who were diagnosed by endoscopic color Doppler ultrasono0graphy (ECDUS) and some cases enrolled in my previous study [19] were included in this study. Clinical manifestations and diagnostic evaluation of ECDUS were analyzed retrospectively.

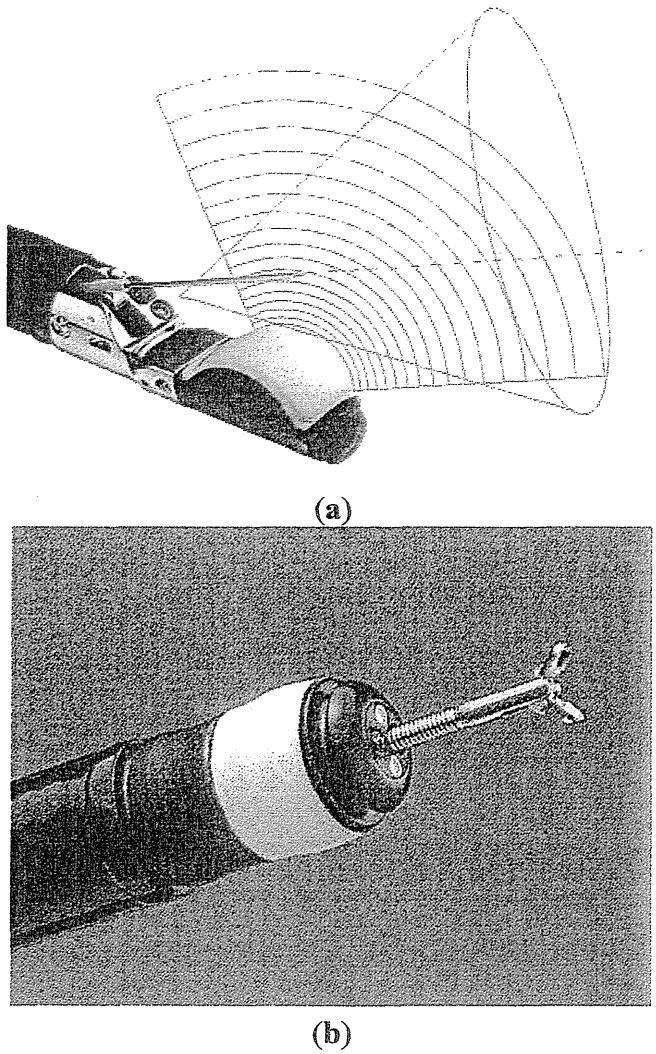
2.2. ECDUS

Hemodynamic evaluation of the gastric varices was performed by ECDUS using a PENTAX FG-36UX (forward-oblique viewing), 7.5 MHz, convex type, which provided 100° images (convex type ECDUS) (Figure 2a) or EG-3630UR (forward viewing), 10 MHz, electronic radial type, which provided 270° images (electronic radial type ECDUS) (Figure 2b) (Pentax Optical, Tokyo, Japan). The HITACHI EUB565 or EUB8500 was used for the display (Hitachi Medical, Tokyo, Japan).

Exploration of gastric varices was conducted by introducing deaerated water from an autoinfuser device through the working channel into the stomach. Evaluation of gastric varices was carried out using ECDUS while the patients remained in a left lateral decubitus position. Initially, identification of gastric varices was made by B-mode scanning followed by color flow mapping. On B-mode scanning, submucosal gastric varices and perigastric collateral veins were obtained as hypoechoic vessels within the gastric wall or in the tissue and spaces exterior to the adventitia of the gastric wall. ECDUS provides a color display of blood flow and evaluates the flow pattern using fast Fourier transform (FFT) analysis. FFT analysis can indicate the flow pattern and calculate the velocity of blood flow. We monitored the color flow images of gastric varices and peri-gastric collateral veins. Velocities were assessed using the pulsed Doppler method, by positioning a sample volume of 1–2 mm in the center of the vessels. The color gain was adjusted so as to eliminate background noise, and the insonation angle was

kept below 60° to minimize ambiguity in measurements of blood flow. The mean velocity of blood flow in the gastric varices was obtained by averaging a total of two tracings of conspicuous points (at least three points) on the gastric varices and was selected as the data showing exceedingly high velocity as the blood velocity of patient. The wall thickness of submucosal varices also was evaluated. The wall thickness was obtained at conspicuous points (at least three points), erosion or red color positive site on the gastric varices, and the data were selected showing exceedingly low thickness as the wall thickness of patient.

Figure 2. (a) PENTAX FG-36UX (forward-oblique viewing), 7.5 MHz, convex type; (b) PENTAX EG-3630UR (forward viewing), 10 MHz, electronic radial type.



(a)

(b)

The absence of a control group of patients with gastric varices due to causes other than splenic vein occlusion is a limitation of this study. The study was performed according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients prior to the procedures. The study was approved by the ethical committee of Sapporo Kosei General Hospital.

3. Results

3.1. Clinical Details

Among the 16 cases, the reasons for endoscopic examination were as follows: hematemesis in 1 case, tarry stool in 3 cases, screening in 4 cases, and confirmation of gastric varices suspected via computed tomography (CT) in 8 cases. According to ultrasound examinations and CT, or angiographic examination, 13 patients had co-existing pancreatic diseases, including 8 with chronic pancreatitis, 4 with cancer of the pancreatic body or tail and 1 with severe acute pancreatitis. Among the 3 remaining patients, 1 had myeloproliferative disease, 1 had advanced gastric cancer, and the third had splenic vein occlusion due to an obscure cause.

3.2. Endoscopic Findings

Endoscopic findings of gastric varices [23] were: variceal form (F) classified as enlarged tortuous (F2) in 12 cases and large, coil-shaped (F3) in 4 cases, and erosion or red color sign of the variceal surface-positive in 4 cases and negative in 12 cases. Three of the 4 cases with erosion or red color sign of the variceal surface had a current history of gastric variceal bleeding.

3.3. ECDUS

Using ECDUS color flow imaging, gastric varices were delineated in all 16 patients. FFT analysis of variceal blood flow showed a continuous wave in all 16 patients, with flow velocities in the gastric varices ranging between 8.6 and 28.6 cm/s (mean 17.1 ± 4.9 cm/s). Figure 3 shows an electronic radial-type ECDUS image of large, coil-shaped gastric varices located between the fundus and the curvatura ventriculi major of the gastric body, which flows as a continuous wave. Endoscopic findings showed enlarged tortuous, erosion-positive gastric varices in a round fundal region with the pancreatic cancer (Figure 4a). In this case, ECDUS demonstrated clearly gastric variceal color flow images of the round fundal region at the center that expand to the curvatura ventriculi major of the gastric body (Figure 4b). All 16 cases diagnosed as gastric varices secondary to splenic vein occlusion showed similar specific findings on ECDUS color flow images. The ECDUS color flow images of gastric variceal flow depicted specific findings of gastric varices secondary to splenic vein occlusion at the round fundal region at the center, with varices expanding to the curvatura ventriculi major of the gastric body.

We compared the velocities of the gastric varices according to variceal form. The mean velocity of the F3 type gastric varices was 23.0 ± 4.0 cm/s (n = 4), while the mean velocity of the F2 type was 15.1 ± 3.4 cm/s (n = 12). The velocities of the F3 type were significantly higher than those of the F2 type ($p < 0.01$). Next, we evaluated the wall thickness of submucosal gastric varices. The gastric wall thickness of the submucosal gastric varices was measured at between 0.8 and 2.0 mm (mean 1.5 ± 0.4 mm). The mean wall thickness of red color sign or erosion-positive varices was 1.1 ± 0.2 mm (n = 4), while the mean thickness of red color sign or erosion-negative varices was 1.7 ± 0.3 mm (n = 12). Thus, the walls of red color sign or erosion-positive varices were significantly thinner than those that were negative ($p < 0.01$).

Figure 3. Endoscopic color Doppler ultrasonography showing a color flow image of gastric varices due to splenic vein occlusion that flow as a continuous wave.

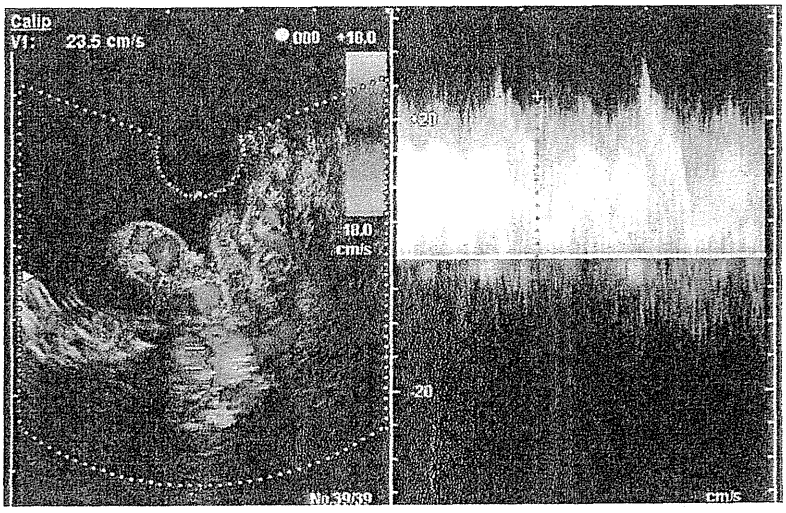
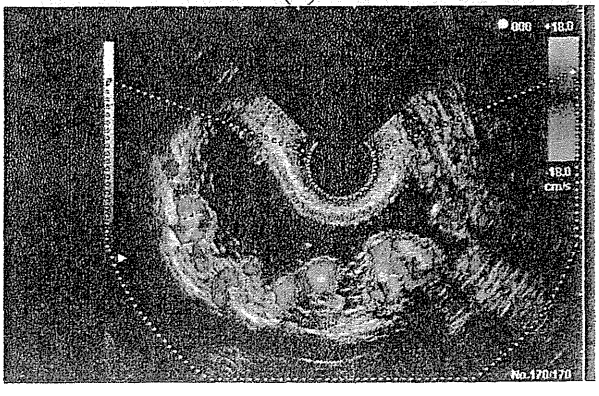


Figure 4. (**a**) Endoscopic findings showing enlarged tortuous, erosion-positive gastric varices in the round fundal region at the center; (**b**) Color flow images from endoscopic color Doppler ultrasonography showing a round fundal region at the center, with varices expanding to the curvatura ventriculi major of the gastric body.

(**a**)



(**b**)

4. Discussion

Gastric varices have been diagnosed by esophago-gastro-duodenoscopy (EGD), a useful modality for observing gastric varices of a certain size and extent. EGD is usually the initial investigation in patients with portal hypertension in order to distinguish between gastric varices and gastric folds, and it has a very sensitive predictive value for variceal hemorrhage [24]. However, cases of red color-positive gastric varices are quite rare and it is difficult to diagnose a high risk of bleeding of gastric varices. Furthermore, EGD is a limited modality for detecting gastric varices, given the depth of the submucosal and extramural collateral veins of gastric varices.

Endoscopic ultrasonography (EUS) has become a useful modality for the diagnosis for esophagogastric varices and is considered most useful for evaluating gastric varices [25–27]. By applying EUS with Doppler capabilities, ECDUS allowed the sonographic visualization of the vessels, as well as evaluation of vascular blood flow and morphology. Using B-mode scanning, submucosal gastric varices and perigastric collateral veins were detected as hypoechoic vessels within the gastric wall, or in tissue and spaces exterior to the adventitia of the gastric wall. The gastric variceal channel and the extension of the gastric body can be observed easily with B mode EUS. In a previous report, Sato *et al.* [28] described the utility of ECDUS in patients with gastric varices. Relative to EUS, ECDUS more clearly delineates the visualization of varices, and the color images of blood flow in vessels allow detailed sonographic visualization of the vessels and evaluation of vascular blood flow for the diagnosis of gastric varices.

Endoscopic evidence is not sufficient to distinguish between gastric varices due to splenic vein occlusion or the gastric fold. Additional images resulting from ECDUS color analysis of gastric variceal flow clearly depicted a round fundal region at the center, with varices that expanded to the curvatura ventriculi major of the gastric body on gastric varices due to splenic vein occlusion. The location of ordinary gastric varices was classified as fundal (located far from the cardiac orifice) and cardiac and fundal (located between the cardiac orifice and the fundus) [23], however, there was no case with varices expanding to the curvatura ventriculi major of the gastric body among the ECDUS findings of ordinary gastric varices [28]. These data provide specific findings that may be regarded as hallmarks of gastric varices due to splenic vein occlusion. FFT analysis was used to evaluate the flow pattern and calculate the velocity of blood flow at gastric varices. ECDUS was used to measure the extent and velocity of blood flow in gastric varices, as well as the thickness of the gastric wall to submucosal gastric varices. In addition to a red-color sign obtained during EGD, ECDUS measurements of blood flow velocity and wall thickness in cases with gastric varices may also be useful for determining risks of variceal bleeding, and these findings may be useful in diagnosing the risk of bleeding of gastric varices due to splenic vein occlusion. However, there is no conclusion regarding the relationship between the ECDUS data and bleeding.

In this study, we have expanded the previous case series [19], and the specific ECDUS findings published in the previous paper are similar to those reported here. Also, ECDUS analysis of variceal velocities and wall thickness in this study are similar to the results in another earlier study [28]. It should be noted that the sample size in this report is small and that investigations involving a larger number of patients will be necessary to confirm the results. At present, although there is no consensus on the treatment of gastric varices secondary to splenic vein occlusion, we suggest that this should be targeted at the underlying diseases.

5. Conclusions

In conclusion, ECDUS color flow images of gastric variceal flow clearly depicted round fundal regions at the center, with varices expanding to the curvatura ventriculi major of the gastric body as the specific findings of gastric varices secondary to splenic vein occlusion.

Conflicts of Interest

The authors declare no conflicts of interest.

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The prospective randomized study on telaprevir at 1500 or 2250 mg with pegylated interferon plus ribavirin in Japanese patients with HCV genotype 1

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Abstract
Background Triple therapy with telaprevir (TVR), pegylated interferon and ribavirin has improved antiviral efficacy in patients with chronic hepatitis C (CH-C). However, the severe adverse effects caused by TVR are important to resolve. In this prospective, randomized, multicenter, open-label study, the antiviral efficacy and safety in the reduced administration of TVR were examined.
Methods A total of 81 CH-C Japanese patients with HCV genotype 1 were randomized into two regimens of TVR 2250 mg (TVR-2250) or 1500 mg (TVR-1500) and treated with triple therapy for 24 weeks.
Results The mean HCV RNA at start, 2 and 4 weeks of treatment were 6.69 ± 0.70, 1.05 ± 0.74, 0.22 ± 0.48 $\log_{10}$ IU/ml in the TVR-2250 group and 6.70 ± 0.62, 1.02 ± 0.62, 0.13 ± 0.41 $\log_{10}$ IU/ml in the TVR-1500 group. The SVR rates were 85 % in both groups (35/41 and 34/40, respectively). There were no patients with viral breakthrough in either group. As for adverse effects, rash more than moderate and severe anemia with <8.5 g/dl of hemoglobin were higher in the TVR-2250 group than in the TVR-1500 group ($p = 0.046$, $p < 0.001$, respectively). The increase in serum creatinine levels and decrease in estimated glomerular filtration rates were higher in the TVR-2250 group than in the TVR-1500 group.
Conclusions The lower dose of TVR (1500 mg/day) can result in similar SVR rates and lower treatment-related adverse effects compared to the higher dose of TVR

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(2250 mg/day) in triple therapy (UMIN: 000007313, 000007330).

Keywords Chronic hepatitis C · Telaprevir · Pegylated interferon plus ribavirin · Dose reduction

Abbreviations

HCV	Hepatitis C virus
IFN	Interferon
Peg-IFN	Pegylated interferon
RBV	Ribavirin
PI	Protease inhibitor
TVR	Telaprevir
SVR	Sustained virologic response
EOT	End of treatment
HCC	Hepatocellular carcinoma
CH-C	Chronic hepatitis C
Hb	Hemoglobin
WBC	White blood cell
RVR	Rapid virologic response
c-EVR	Complete early virologic response
ETR	End of treatment response
SMV	Simeprevir

Introduction

Antiviral therapy for patients with chronic hepatitis C virus (HCV) genotype 1 infection has changed from interferon (IFN) monotherapy to dual therapy with pegylated IFN (Peg-IFN) and ribavirin (RBV) and then to triple therapy with protease inhibitor (PI), Peg-IFN and RBV [1]. The clinical trials of triple therapy with telaprevir (TVR), which is a first-generation PI, Peg-IFN and RBV have reported that addition of TVR leads to a substantial improvement in sustained virologic response (SVR), defined as an undetectable HCV RNA at 24 weeks after the end of treatment (EOT), compared to Peg-IFN and RBV among naïve patients and re-treatment patients [2–9]. However, severe adverse effects caused by TVR, such as severe anemia, rash and gastrointestinal disorder, were also reported [2, 3, 8, 9]. Generally, older patients and patients with advanced liver fibrosis have greater risk of hepatocellular carcinoma (HCC) and, therefore, should be treated with antiviral therapy as early as possible in order to eliminate HCV. However, adverse effects caused by TVR in those patients may lead to serious complications, such as death [10].

A phase 1b, placebo-controlled, double-blinded study conducted in Europe evaluated the antiviral activity, pharmacokinetics and safety of TVR [11]. That study examined the antiviral activity of TVR for 14 days at 450 or 750 mg every 8 h or 1250 mg every 12 h. The highest trough plasma drug concentrations, and the greatest HCV RNA reduction were measured in the 750-mg-dose group. The most frequent adverse effects were headache, diarrhea, fatigue, nausea and dry skin, and there were no severe adverse effects and no dosing interruptions. As a result, the regimen of 750 mg of TVR every 8 h (total 2250 mg/day) was selected. Subsequently, a phase 2 study for triple therapy with TVR, Peg-IFN and RBV was conducted in Europe and the United States [2–4]. However, a dose-finding study of TVR in triple therapy was not conducted in these phase 2 studies. The dose of TVR in triple therapy was determined based on the TVR monotherapy study. On the other hand, in Japan, the phase 1, open-label, two-arm study of TVR at 500 or 750 mg every 8 h with Peg-IFN alfa-2b and RBV was conducted using 20 chronic hepatitis C (CH-C) patients [12, 13]. The pharmacokinetic parameters such as the area under the plasma concentration time curve from 0 to 8 h (AUC_{0-8h}), the maximum plasma concentration (C_{max}) and the trough plasma concentration (C_{trough}) of TVR were higher in the 750-mg dose group than in the 500-mg dose group, and there was no difference in adverse effect between these two groups. As a result, a regimen of 750 mg of TVR every 8 h (total 2250 mg/day) was selected, although HCV RNA reduction was similar in both groups.

In the phase 3 study of triple therapy with TVR, Peg-IFN and RBV, similar SVR rates were observed in clinical trials in Europe, the United States and Japan (naive 69–75 vs. 73 %, treatment-relapse, 83–88 vs. 88 %) [5–9]. Whereas the rate of discontinuation of all drugs and the rate of discontinuation of TVR were 7–10 % and 7–12 % in Europe and the United States, those rates were higher at 17 % and 19 % in Japan. As for the severe adverse effects, rash and anemia were more frequent in Japan compared to Europe and the United States (rash 12 % vs. <1 %; anemia 11 % vs. 2 %). More adverse effects with triple therapy in Japanese patients may result from excessive doses of TVR because the dosage of TVR is constant, and Japanese people tend to weigh less than Western people. Alternatively, Asian people including Japanese can cause severe adverse effects compared to Western people. Therefore, the triple therapy with reduced TVR, Peg-IFN and RBV has the potential to improve safety of Japanese patients with CH-C.

In this multicenter, randomized study, we examined the antiviral efficacy and safety in triple therapy after administration of two TVR dosages: 1500 and 2250 mg/day.

Patients and methods

Patients

The current study was a prospective, randomized, multicenter, open-label study conducted by Osaka University

Hospital and other institutions participating in the Osaka Liver Forum. A total of 81 CH-C patients were enrolled in this study between December 2011 and December 2012.

Eligible patients were 20 years of age and older, had chronic HCV genotype 1 infection with a viral load of more than 10^5 IU/ml, did not have co-infection with hepatitis B or anti-human immunodeficiency virus and had an absolute neutrophil count of 1500/mm^3 or more, a platelet count of 10×10^4/mm^3 or more, and a hemoglobin (Hb) level of 12 g/dl or more. The patients were accepted regardless of history of IFN treatment. The patients were excluded if they had decompensated cirrhosis, HCC or other forms of liver disease (alcohol liver disease, autoimmune hepatitis), an experience with splenectomy or partial spleen embolization, chronic renal failure, depression or immunodeficiency. This study was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki amended in 2002 and was approved by the ethics commission of Osaka University Hospital and independent or institutional review boards of all study centers (UMIN000007313, 000007330). All patients provided written informed consent before participating in the study.

Study design

This study was a randomized, open-label trial. Patients were stratified according to gender and age (<60 vs. ≥60 years old) and were randomly assigned to one of two groups. The TVR-1500 group received 1500 mg/day of TVR (TELAVIC; Mitsubishi Tanabe Pharma, Osaka, Japan), Peg-IFN alfa-2b (PEGINTRON; MSD, Tokyo, Japan) and RBV (REBETOL; MSD) for 12 weeks, followed by Peg-IFN alfa-2b and RBV for 12 more weeks. The TVR-2250 group received 2250 mg/day of TVR, Peg-IFN alfa-2b and ribavirin for 12 weeks, followed by Peg-IFN alfa-2b and RBV for 12 more weeks. The randomization was performed at a 1:1 ratio between the two groups.

TVR was administered orally at a dose of 500 or 750 mg every 8 h after food. Peg-IFN alfa-2b was administered subcutaneously once a week at a dose of 60–150 μg/kg based on body weight (body weight 35–45 kg, 60 μg; 46–60 kg, 80 μg; 61–75 kg, 100 μg; 76–90 kg, 120 μg; 91–120 kg, 150 μg), and RBV was administered orally twice a day at a total dose of 600 to 1000 mg/day based on body weight (body weight <60 kg, 600 mg; 60–80 kg, 800 mg; >80 kg, 1000 mg), according to a standard treatment protocol for Japanese patients. In principle, the patients were treated with TVR, Peg-IFN and RBV for 12 weeks, followed by Peg-IFN and RBV for 12 weeks. If patients had detectable HCV RNA at 12 weeks or any time during weeks 13 through 20, they were not permitted to complete the remainder of the assigned duration of therapy.

Dose modification

Dose modification followed the manufacturers' drug information. The initial dose of RBV was reduced by 200 mg per day when the Hb level was <13 g/dl at baseline. The dose of Peg-IFN alfa-2b was reduced to 50 % of the assigned dose if the white blood cell (WBC) count declined to <1500/mm^3, the neutrophil count to <750/mm^3, or the platelet count to $<8 \times 10^4$/mm^3. RBV was also reduced from 1000 to 600 mg, from 800 to 600 mg, or from 600 to 400 mg if the Hb level decreased to <12 g/dl, and the dose was reduced by an additional 200 mg per day when the Hb level was <10 g/dl. The dose of RBV was also reduced by 200 mg per day if the Hb level dropped more than 1 g/dl within a week, and this level was <13 g/dl. TVR, Peg-IFN alfa-2b and RBV were withdrawn or interrupted if the WBC count declined to <1000/mm^3, the neutrophil count to <500/mm^3, the platelet count to $<5 \times 10^4$/mm^3, or the Hb level to <8.5 g/dl. TVR was reduced from 2250 to 1500 mg, from 1500 to 750 mg according to adverse effects of TVR, such as rash, anemia, intestinal disorder and increase of serum creatinine levels by physician's decision. The use of erythropoietin was not allowed for elevating the Hb level. In the case of drug interruption with TVR or Peg-IFN and RBV, if peripheral blood finding or adverse effects subsided, resumption of treatment was allowed.

Histological evaluation

Pre-treatment liver biopsies were conducted within 6 months of the start of the combination therapy. Histopathological interpretation of the specimens was performed by experienced liver pathologists who had no clinical, biochemical or virological information. The histological appearances, activity and fibrosis were evaluated according to the METAVIR histological score [14].

Virologic assessment and definition of viral response

Serum HCV RNA levels were quantified with the COBAS Taqman HCV test, version 2.0 (detection range 1.2–7.8 log IU/ml; Roche Diagnostics, Branchburg, NJ). Serum HCV RNA level was assessed before treatment, at weeks 2, 4, 8, 12, 16, 20 and 24 during treatment and 24 weeks after the therapy. A rapid virologic response (RVR) was defined as undetectable serum HCV RNA at week 4, a complete early

virologic response (c-EVR) as undetectable serum HCV RNA at week 12 and an EOT response (ETR) as undetectable serum HCV RNA at EOT. An SVR was defined as an undetectable serum HCV-RNA level at 24 weeks after the EOT. Relapse was defined as an undetectable serum HCV RNA level at the EOT but a detectable amount after the EOT. Non-response was defined as a detectable HCV RNA level during therapy. Breakthrough was defined as quantifiable HCV RNA after undetectable HCV RNA during therapy.

Safety assessment

Chemical and hematologic assessments and a safety assessment were performed every week during the first 12 weeks of treatment and every 4 weeks from week 12 to week 24 of treatment. At each visit, data on adverse effects were collected and physical examinations were performed, if clinically indicated.

Statistical analysis

Baseline continuous variables were expressed as the mean ± standard deviation or median and categorical variables as frequencies. The virologic response was evaluated in intention-to-treat set. Differences between the two groups were assessed by a Chi Square test or a Mann–Whitney U test and a t test. The cumulative incidence of adverse effects was assessed with the Kaplan–Meier method and a log-rank test. A p value < 0.05 was considered significant. Statistical analysis was conducted with SPSS version 19.0 J (IBM, Armonk, NY, USA).

Results

Patients

Eight-one Japanese patients underwent randomization and received treatment (Fig. 1). The baseline characteristics of the patients were similar between the two treatment groups except for the TVR dose (Table 1). The mean TVR dose per body weight was significantly higher in the TVR-2250 group than in the TVR-1500 group. Among the patients with non-response, 4 patients were partial-responder with ≥ 2 $\log_{10}$ IU/ml of HCV RNA decrease in previous Peg-IFN and RBV and no patients were null-responder with <2 $\log_{10}$ IU/ml of HCV RNA decrease in previous Peg-IFN and RBV in the TVR-2250 group (one patient was unknown). There were 3 partial-responders and 4 null-responders were in the TVR-1500 group (one patient was unknown).

Virologic response

The mean HCV RNA at start, 2 and 4 weeks of treatment were 6.69 ± 0.70, 1.05 ± 0.74 and 0.22 ± 0.48 $\log_{10}$ IU/ml, respectively, in the TVR-2250 group and 6.70 ± 0.62, 1.02 ± 0.62 and 0.13 ± 0.41 $\log_{10}$ IU/ml, respectively, in the TVR-1500 group, and there was no significant difference (Fig. 2a). As for naïve patients, similar decreases in HCV RNA were attained in both groups (Fig. 2b). There was no significant difference in RVR, cEVR, ETR and SVR rates in both groups (Fig. 3a). According to history of IFN treatment, there was no significant difference in SVR rates in both groups (Fig. 3b). Two patients with non-SVR in the TVR-1500 group were null-responders in previous Peg-IFN and RBV.

Drug reduction and discontinuation

All three drugs were discontinued until 12 weeks in 4 patients (10 %) in both groups. TVR was discontinued in 10 patients (24 %) of the TVR-2250 group and in 9 patients (23 %) of the TVR-1500 group. The main reasons for discontinuation of TVR were anemia, rash, gastrointestinal disorder, general fatigue, hyperbilirubinemia and renal dysfunction. Table 2 shows the proportion of patients with dose reduction and discontinuation due to adverse effects. Although the discontinuation rates of TVR were similar in both groups, the number of patients without dose reduction or discontinuation of TVR was larger in the TVR-1500 group than in the TVR-2250 group (68 vs. 32 %, $p = 0.001$). RBV was reduced at 93 % in the TVR-2250 group and 88 % in the TVR-1500 group, and the number of patients without dose reduction or discontinuation of TVR was low in both groups (7, 13 %, respectively). On the other hand, the number of patients without dose reduction or discontinuation of Peg-IFN was larger in the TVR-1500 group than in the TVR-2250 group (24 vs. 48 %, $p = 0.03$).

Adverse effects

Rash was common in the TVR-2250 group (68 %: grade 1, 28 %; grade 2, 35 %; grade 3, 5 %) and in the TVR-1500 group (58 %: grade 1, 36 %; grade 2, 22 %; grade 3, 0 %). The cumulative occurrence of rash more than moderate was significantly higher in the TVR-2250 group than in the TVR-1500 group ($p = 0.046$, Fig. 4a). Anemia, defined as a decline of more than 3 g/dl of Hb, occurred in 98 % of the TVR-2250 patients and 85 % of the TVR-1500 patients. The decreases of Hb from baseline were significantly greater in the TVR-2250 group than in the TVR-1500 group at 4 and 8 weeks of treatment (4 weeks, 3.4 ± 1.1 vs. 2.8 ± 1.3 g/dl, $p = 0.03$; 8 weeks, 4.5 ± 1.4 vs. 3.4 ± 1.3 g/dl, $p = 0.001$). Severe anemia, defined as

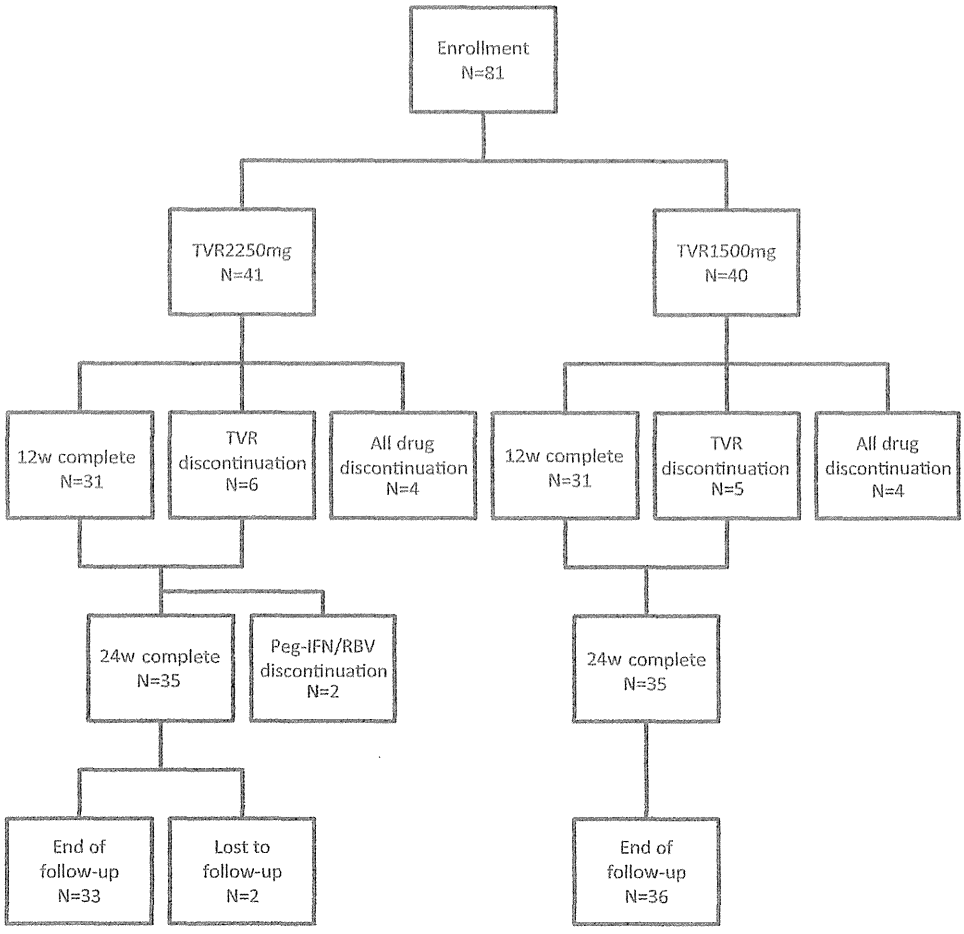

Fig. 1 The patients flow of this study. The patients lost to follow-up were determined as non-SVR

<8.5 g/dl of Hb, affected 39 % of the TVR-2250 patients and 8 % of the TVR-1500 patients. The cumulative occurrence of severe anemia was significantly higher in the TVR-2250 group than in the TVR-1500 group ($p = 0.001$, Fig. 4b). The mean minimum Hb was significantly lower in the TVR-2250 group than in the TVR-1500 group (8.7 ± 1.4 vs. 10.1 ± 1.4 g/dl, $p < 0.001$). As for renal dysfunction, the mean serum creatinine levels were higher (Fig. 5a) and the mean estimated glomerular filtration rates (eGFR) were lower in the TVR-2250 group than in the TVR-1500 group during weeks 1–4 (Fig. 5d). A stratified analysis by age clearly shows that these abnormalities of the serum creatinine levels and eGFR were more marked in older patients (≥60 years old) than younger patients (<60 years old) (Fig. 5b, c, e, f).

Discussion

The dose of TVR in triple therapy, a regimen of 750 mg of TVR every 8 h (total 2250 mg/day) was selected all over the world. However, since Western people weigh more than Asian people including Japanese (BMI in phase 3 trials, 26–27 kg/m^2 in Western countries, 22–23 kg/m^2 in Japan) [5–9], the lighter-build Japanese patients may receive an excess of TVR. Therefore, we conducted the first randomized, multicenter study to evaluate the antiviral efficacy and safety after administration of TVR at 750 mg or 500 mg every 8 h with Peg-IFN alfa-2b and RBV.

As for antiviral effect, the HCV RNA reduction for the first 4 weeks of treatment was almost the same in the TVR-2250 group and the TVR-1500 group. Moreover, in stratified analysis for the effect of previous IFN treatment, the SVR rates were almost the same in both TVR groups among the naïve patients and relapsers. The SVR rates in patients with non-response were 100 % (5/5) in the TVR-2250 group and 75 % (6/8) in the TVR-1500 group. In triple therapy with TVR, Peg-IFN and RBV, it has been clearly shown from the phase 3 REALIZE study that the SVR rates were higher in partial-responders than in null-responders [7]. Among the patients with non-response in this study, 4 partial-responders were included in the TVR-