

probably attributed to the fact that the subjects of the studies are the patients who had mild disease and needed no antiviral therapy. TE would be a useful tool to detect the patients with progressive fibrosis for the physicians in the follow-up of the patients with chronic viral hepatitis.

The results of TE were not confirmed by the results of liver biopsies in the articles reviewed. The absence of comparison with biopsies is the limitations of these studies.

ESTIMATION OF PROGNOSIS OF HEPATITIS

THE RISK OF hepatocellular carcinoma (HCC) or bleeding from esophageal varices is high in patients with advanced fibrosis.^{39,40} Thus it is important to detect advanced fibrosis early and start the search for HCC and varices in order to treat them in early stage or before bleeding.

A meta-analysis of performance of TE for fibrosis staging demonstrated that the mean AUROC for cirrhosis was 0.94 (95% CI, 0.93–0.95) and an adjusted AUROC of 0.99 and that the optimal cut-off value for cirrhosis suggested from the summary ROC techniques was 13.01 kPa.¹⁷

Piscaglia *et al.* studied 90 patients with chronic liver disease with ARFI.²⁹ The AUROC for the diagnosis of cirrhosis was 0.941 with 1.75 m/s as the optimal cut-off (sensitivity 93.0%; specificity 85.1%).

Lupsor *et al.* studied 112 patients with chronic hepatitis C with ARFI.²³ The AUROC for the diagnosis of cirrhosis was 0.936 with 2 m/s as the optimal cut-off (sensitivity 80.0%; specificity 95.45%).

Sporea *et al.* studied 71 patients with chronic liver diseases with ARFI.²⁴ The AUROC for the diagnosis of cirrhosis was 0.868 with 1.8 m/s as the optimal cut-off (sensitivity 100%; specificity 77%).

Toshima *et al.* studied 79 patients with chronic liver diseases with ARFI.²⁵ The AUROC for the diagnosis of cirrhosis was 0.87 with 1.79 m/s as the optimal cut-off (sensitivity 86%; specificity 79%).

Ebinuma *et al.* studied 59 patients with chronic viral hepatitis with ARFI.²⁶ The AUROC for the diagnosis of cirrhosis was 0.854 with 1.88 m/s as the optimal cut-off (likelihood ratio 4.55).

The summary of investigations of ARFI for assessment of cirrhosis is shown in Table 1.^{10,21–29}

Friedrich-Rust *et al.* studied 79 patients with chronic viral hepatitis with real-time elastography.¹¹ The cut-off value of elastic ratio and AUROC for cirrhosis was

111.75 and 0.69, respectively (sensitivity 29.2%; specificity 90.7%).

Koizumi measured LS with real-time tissue elastography in 70 patients with chronic hepatitis C.²⁰ The cut-off value of elastic ratio and AUROC for cirrhosis were 3.93 and 0.95, respectively (sensitivity 90.9%; specificity 91.5%).

Stefanescu *et al.* compared the performance of common serum fibrosis scores and TE in diagnosing esophageal varices in 231 cirrhosis patients.⁴¹ The Lok Score⁴² was the best among all the serum scores for diagnosing the varices; cut-off value for large varices is 0.8 (positive predictive value 45.5%, negative predictive value 86.4% and diagnostic accuracy 67.72%). The cut-off value of LS for large varices is 30.8 kPa (positive predictive value 47.3%, negative predictive value 81% and diagnostic accuracy 68.32%). Using both tests simultaneously, the presence of large varices was predicted with a diagnostic accuracy of 78.12%, obtaining an increment in negative predictive value and negative likelihood ratio up to 93.67% and 0.21, respectively.

Jung *et al.* investigated the usefulness of LS by TE as a predictor of HCC development in 1130 patients with chronic HBV infection.⁴³ During the follow-up period (median, 30.7 months; range, 24.0–50.9 months), HCC developed in 57 patients (2.0% per 1 person-year). The 1-, 2-, and 3-year cumulative incidence rates of HCC were 0.80%, 3.26%, and 5.98%, respectively. On multivariate analysis, together with old age, male sex, heavy alcohol consumption (>80 g/day), serum albumin, and hepatitis B e antigen positivity, patients with a higher LS (>8 kPa) were at a significantly greater risk of HCC development, with the following hazard ratios: 3.07 (95% confidence interval [CI], 1.01–9.31; $P=0.047$) for LS 8.1–13 kPa; 4.68 (95% CI, 1.40–15.64; $P=0.012$) for LS 13.1–18 kPa; 5.55 (95% CI, 1.53–20.04; $P=0.009$) for LS 18.1–23 kPa; and 6.60 (95% CI, 1.83–23.84; $P=0.004$) for LS > 23 kPa.

Masuzaki *et al.* investigated the relationship between LS and HCC presence in the cross-sectional study.⁴⁴ LS was measured in chronic hepatitis C patients (85 with HCC and 180 without) by TE. Multivariate analysis showed that HCC presence was significantly associated with LS ($P<0.0001$) along with age, male, and α -fetoprotein concentration. AUROC was 0.805, 0.741, 0.714, 0.673, 0.670, and 0.654 for LS, α -fetoprotein, albumin, prothrombin activity, aspartate aminotransferase (AST)-platelet ratio index, and platelet count, respectively. Stratum-specific likelihood ratio for HCC presence by LS was 0.22 (95% CI: 0.11–0.42) in

<10 kPa, 0.73 (0.39 to 1.39) in 10.1 to 15 kPa, 1.30 (0.80 to 2.12) in 15.1 to 25 kPa, and 5.0 (2.96 to 8.47) in >25 kPa.

Masuzaki *et al.* investigated the relationship between baseline LS and HCC development prospectively among 866 patients with chronic hepatitis C.⁴⁵ During the follow-up period (mean, 3.0 years), HCC developed in 77 patients (2.9% per 1 person-year). The cumulative incidence rates of HCC at 1, 2, and 3 years were 2.4%, 6.0%, and 8.9%, respectively. Adjusting for other significant factors for HCC development, patients with higher LS were revealed to be at a significantly higher risk, with a hazard ratio, as compared to LS < or =10 kPa, of 16.7 (95% CI, 3.71–75.2; $P < 0.001$) when LS 10.1–15 kPa, 20.9 (95% CI, 4.43–98.8; $P < 0.001$) when LS 15.1–20 kPa, 25.6 (95% CI, 5.21–126.1; $P < 0.001$) when LS 20.1–25 kPa, and 45.5 (95% CI, 9.75–212.3; $P < 0.001$) when LS > 25 kPa.

Thus TE, real-time elastography and ARFI are useful for diagnosis of cirrhosis and prediction of development of varices or HCC.

CAN LIVER STIFFNESS REPLACE LIVER BIOPSY?

TRANSIENT ELASTOGRAPHY, ARFI and real-time elastography are the methods with very good or excellent diagnostic accuracy for the assessment of liver fibrosis stage. They do not provide information on inflammatory activity, steatosis, iron deposition or other findings in liver biopsy. Even on account of fibrosis stage, these non-invasive methods do not give us the estimation completely corresponding to that of liver biopsy. In addition, the values of LS might be affected by factors other than fibrosis stage, for example, inflammatory activity^{9,18} and intrahepatic pressure.⁴⁶ However they provide us useful clinical information, which liver biopsy has been providing us as described in the present article, such as appropriate time to start antiviral therapy, prediction of response to antiviral therapy, evaluation of effects of antiviral therapy, assessment of natural course of hepatitis and estimation of prognosis of hepatitis. Recently non-invasive methods for assessment of inflammatory activity,⁴⁷ steatosis^{48,49} and iron deposition⁵⁰ in the liver have been developed. Such as ActiTest,⁴⁷ SteatoTest,⁴⁹ and MR imaging for quantification of fat⁴⁸ and iron contents⁵⁰ in liver provide the information other than fibrosis derived from liver biopsy. Thus in the near future, non-invasive methods will replace liver biopsy.

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Prevalence of Hepatitis C Virus Genotype 1a in Japan and Correlation of Mutations in the NS5A Region and Single-Nucleotide Polymorphism of Interleukin-28B With the Response to Combination Therapy With Pegylated-Interferon-Alpha 2b and Ribavirin

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Hepatitis C virus (HCV) genotype 1a is rare in Japanese patients and the clinical characteristics of this genotype remain unclear. The interferon (IFN) sensitivity-determining region (ISDR) and single-nucleotide polymorphisms (SNPs) of interleukin-28B (IL28B) among patients with HCV genotype 1b are associated with IFN response, but associations among patients with genotype 1a are largely unknown. This study investigated the clinical characteristics of genotype 1a and examined whether genomic heterogeneity of the ISDR and SNPs of IL28B among patients with HCV genotype 1a affects response to combination therapy with pegylated-IFN- α 2b and ribavirin. Subjects comprised 977 patients infected with HCV genotype 1, including 574 men and 412 women (mean age, 55.2 ± 10.6 years). HCV was genotyped by direct sequencing of the 5'-untranslated region and/or core regions and confirmed by direct sequencing of the NS5A region. HCV genotypes 1a ($n = 32$) and 1b ($n = 945$) were detected. Twenty-three (71.9%) of the 32 patients with genotype 1a were patients with hemophilia who had received imported clotting factors. Prevalence of genotype 1a after excluding patients with hemophilia was thus 0.9%. Of the 23 patients with genotype 1a who completed IFN therapy, 11 (47.8%) were defined as achieving sustained virological response. Factors related to sustained virological response by univariate analysis were IL28B and ISDR. In conclusion,

HCV genotype 1a is rare in Japan. The presence of IL28B genotype TT, and more than two mutations, in the ISDR are associated with a good response to IFN therapy in patients with HCV genotype 1a. *J. Med. Virol.* **84:438–444, 2012.** © 2012 Wiley Periodicals, Inc.

KEY WORDS: hepatitis C virus; genotype 1a; NS5A; IL 28B; interferon

INTRODUCTION

Hepatitis C virus (HCV) is a member of the Flaviviridae family and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma [Seeff, 2002]. HCV infection is a significant global health problem, affecting 170 million individuals worldwide. HCV can be divided into six genotypes and several subtypes according to genomic heterogeneity [Simmonds et al., 2005]. Each genotype shows a unique distribution and clinical characteristics such

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as interferon (IFN) responsiveness [Ghany et al., 2009]. HCV genotypes 1b, 2a, and 2b are the major types encountered in Japan [Enomoto et al., 1990; Hayashi et al., 2003]. Genotype 1a is common worldwide, but is rare in Japan except among individuals with hemophilia who have received imported clotting factors [Fujimura et al., 1996; Otagiri et al., 2002; Hayashi et al., 2003]. The prevalence and clinical characteristics, including IFN responsiveness, of Japanese patients with HCV genotype 1a are unclear. HCV NS5A protein reportedly includes a domain associated with IFN response. This domain, located in the NS5A region of HCV genotype 1b, is closely associated with response to IFN therapy and is known as the IFN sensitivity-determining region (ISDR) [Enomoto et al., 1996]. IFN acts to inhibit viral replication by inducing double-stranded RNA-dependent protein kinase (PKR). The ISDR is located at the 5' end of the PKR-binding domain and is inhibited by PKR in vitro [Gale et al., 1998]. ISDR heterogeneity of genotype 1b is thus an important factor that may affect response to IFN [Enomoto et al., 1996; Nakano et al., 1999; Pascu et al., 2004; Hayashi et al., 2011a]. Several studies have reported a relationship between ISDR and IFN responsiveness among patients with HCV genotype 1a [Hofgärtner et al., 1997; Zeuzem et al., 1997; Kumthip et al., 2011; Yahoo et al., 2011]. However, this remains controversial for genotype 1a, and the utility of ISDR sequences for predicting IFN responsiveness has not been investigated for HCV genotype 1a in Japan due to the rarity of this genotype. Both genetic heterogeneity of the HCV genome and host genetics contribute to IFN responsiveness. Several genome-wide association studies have thus been performed to clarify host factors associated with IFN responsiveness, revealing that interleukin-28B (IL28B) polymorphisms are strongly associated with response to IFN therapy [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Thomas et al., 2009]. Combined use of the single-nucleotide polymorphisms (SNPs) of IL28B and amino acid substitutions in the core region and ISDR could thus improve the prediction of response to IFN in patients with HCV genotype 1b [Akuta et al., 2011; Hayashi et al., 2011b; Kurosaki et al., 2011]. However, the effects of a combined evaluation of the SNPs of IL28B and amino acid substitutions in the ISDR in patients with HCV genotype 1a on IFN response are unclear. The aim of the present study was to determine whether genomic heterogeneity of the ISDR and SNPs of IL28B among patients with HCV genotype 1a affect response to combination therapy with pegylated-IFN- α 2b and ribavirin.

PATIENTS AND METHODS

A total of 977 patients (569 men, 408 women) with chronic hepatitis C genotype 1 and high viral load (<100 KIU/ml) who were treated at Nagoya University Hospital and affiliated hospitals were enrolled in

this study. Mean age of patients was 55.1 ± 12.2 years (range: 18–75 years). None of the patients had a history of chronic alcohol abuse, autoimmune disease, or metabolic disease. Patients with active intravenous drug use and immigrants were excluded from this study. The core region (aa 30–110) and ISDR (aa 2,209–2,248) of HCV were examined by direct sequencing. SNPs of IL28B (rs8099917) were identified using a real-time polymerase chain reaction (PCR) system. Patients received subcutaneous injections of pegylated-IFN- α 2b (1.5 μ g/kg) once each week along with oral ribavirin (600 mg/day for patients <60 kg, 800 mg/day for 60–80 kg, 1,000 mg/day for >80 kg) for 48 weeks. Patients who became negative for HCV-RNA between 16 and 36 weeks after initiating IFN treatment had the IFN treatment extended to 72 weeks, in accordance with Japanese guidelines [Kumada et al., 2010]. HCV-RNA levels in serum samples were examined at 12 weeks, at the end of IFN therapy, and at 6 months after the end of treatment. Serum was stored at -80°C for virological examination at pretreatment. Early virological response was defined as HCV-negative status at 12 weeks. Patients who were persistently negative for serum HCV-RNA at 24 weeks after withdrawal of IFN treatment were considered to show sustained virological response. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Virological Analysis

HCV-RNA quantitative viremia load was determined by PCR. HCV was genotyped by direct sequencing of the 5'-untranslated region and/or core regions as described previously and confirmed by direct sequencing of the NS5A region [Otagiri et al., 2002; Dal Pero et al., 2007; Hayashi et al., 2011a]. Genotypes were classified according to the nomenclature proposed by Simmonds et al. [2005]. Direct sequencing of the core and NS5A-ISDR regions was performed as reported previously [Dal Pero et al., 2007; Hayashi et al., 2011a]. In brief, RNA was extracted from 140 μ l of serum using a commercial kit (QIAamp Viral RNA Kit; Qiagen, Valencia, CA) and dissolved in 50 μ l of diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligos and random hexamer primers with a commercial kit (iScript cDNA Synthesis Kit; Bio-Rad, Hercules, CA). The HCV core region and NS5A-ISDR were amplified by nested PCR. In brief, each 50- μ l PCR reaction mixture contained 100 nM of each primer, 1 ng of template cDNA, 5 μ l of GeneAmp 10 \times PCR buffer, 2 μ l of dNTPs, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primers for the core region were: sense, 5'-GGGAGGTCTCGTAGACCGTGCAC-CATG-3' and antisense, 5'-GAGMGGKATRTACCC-CATGAGRTCGGC-3'. Primers for the NS5A-ISDR were: sense, 5'-GCCTGGAGCCCTTGTAGTC-3' and

TABLE I. Clinical Characteristic of Patients With HCV Genotype 1a

	N = 32
Age (y.o.)	36.4 ± 2.2
Sex: male/female	28/4
AST (IU/L)	48.8 ± 33.6
ALT (IU/L)	64.6 ± 57.8
Platelet (10 ⁴ /μl)	18.8 ± 6.0
HCV RNA level (KIU/ml)	2607.4 ± 3072.2
Source (clotting factor/BTF/unknown)	23/2/7

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

antisense, 5'-CTGCGTGAAGTGGTGAATAC-3'. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The second PCR was performed using the same reaction buffer with the first-round PCR product as template, and the following sets of primers: for the core region, sense primer 5'-AGACCGTGCACCATGAGCAC-3' and antisense 5'-TACGCCGGGGTCAKTRGGGCCCA-3'; and for the NS5A-ISDR, sense 5'-TGTTTCCCCACGCACTAC-3' and antisense 5'-TGATGGCAGTTTT-TGTTCTTC-3'. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers using a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit; Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems).

Genotyping Analysis

Detection of SNPs for IL28B (rs8099917) was conducted using a real-time PCR system. In brief, genomic DNA was extracted from 150 μl of whole blood with a commercial kit (QIAamp DNA Blood mini Kit; Qiagen) and dissolved in 50 μl of diethylpyrocarbonate-treated water. DNA (10 ng) was used for PCR and genotyping of IL28B SNP (rs8099917) was performed by TaqMan allelic discrimination (ABI-Prism 7300 SDS software; Applied Biosystems) with TaqMan SNP Genotyping Assays provided by Applied Biosystems (C_11710096_10).

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The paired *t*-test was used to analyze differences in variables. A value of *P* < 0.05 was considered statistically significant. Statview 5.0 software (SAS Institute, Cary, NC) was used for all analyses.

RESULTS

Thirty-two of the 977 patients (3.3%) were infected by genotype 1a. Clinical characteristics of patients with genotype 1a are summarized in Table I. Twenty-three cases involved patients with hemophilia who had received imported clotting factors. The prevalence of genotype 1a after excluding patients with hemophilia was 0.9%. A comparison of clinical characteristics according to hemophilia status is shown in Table II. No significant differences were apparent among the two groups. Differences in clinical characteristics between genotypes 1a and 1b are shown in Table III. Males were more frequent among patients with genotype 1a (87.5%) than among those with genotype 1b (57.2%), as the majority of patients with genotype 1a were young male patients with hemophilia. Sequence alignments of the core region at codons 71 and 90 showed arginine and cysteine, respectively, in all patients. The HCV core region of genotype 1a was thus well-conserved, with no significant mutations at codons 71 or 90. This is not similar to previous findings for genotype 1b [Akuta et al., 2005, 2011; Hayashi et al., 2011a,b; Kurosaki et al., 2011]. Alignment of the amino acid sequence for NS5A-ISDR is shown in Figure 1. The sequence of the HCV-1 strain was defined as the consensus sequence of genotype 1a, and the number of mutations to the chosen consensus sequence in ISDR was used to analyze the ISDR system. Sequences of the HCV-1 strain and HCV-1 strain with only one amino acid substitution were defined as wild-type, while ISDR sequences with more than two amino acid substitutions were defined as mutant-type. Twenty-seven strains were defined as wild-type and 5 strains were defined as mutant-type. IL28B genotypes could be obtained for 25 patients, and IL28B alleles were TT (n = 14) and TG (n = 11). Twenty-three patients received pegylated-IFN-α2b plus ribavirin therapy. Twenty patients were treated for 48 weeks, and 1 patient was treated for 72 weeks. Two patients were withdrawn at 24 weeks due to a

TABLE II. Clinical Characteristic According to Hemophilia

	Patients with hemophilia (N = 23)	Patients without hemophilia (N = 9)	<i>P</i> -value
Age (y.o.)	37.1 ± 9.2	37.1 ± 16.3	0.9966
Sex: male/female	22/1	6/3	0.0572
AST (IU/L)	51.2 ± 34.8	41.9 ± 30.9	0.5072
ALT (IU/L)	68.2 ± 55.8	54.0 ± 66.1	0.5566
Platelet (10 ⁴ /μl)	18.4 ± 6.8	19.8 ± 3.0	0.5602
HCV levels (KIU/ml)	2599.6 ± 3108.0	2630.0 ± 3176.5	0.9812

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

TABLE III. Clinical Characteristic According to Genotypes

	Genotype 1a (N = 32)	Genotype 1b (N = 945)	P-value
Age (y.o.)	36.4 ± 2.2	55.9 ± 11.6	0.0001
Sex: male/female	28/4	546/408	0.0004
Patients with hemophilia	23	4	0.0001
AST (IU/L)	48.8 ± 33.6	59.9 ± 45.0	0.1745
ALT (IU/L)	64.6 ± 57.8	64.6 ± 57.8	0.9894
Platelet (10 ⁴ /μl)	18.8 ± 6.0	17.2 ± 6.0	0.0918
HCV levels (KIU/ml)	2607.4 ± 3072.2	2011.5 ± 1453.8	0.0642

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus.

lack of response to IFN therapy. Frequency of early virological response, characterized by undetectable HCV at 12 weeks, was 30.4% (7/23). Virological response rate at the end of treatment was 47.8% (11/23). Finally, 11 of 23 patients (47.8%) achieved sustained virological response. Clinical characteristics were compared between patients who achieved sustained virological response and patients who did not (Table IV), revealing significant differences in two factors on univariate analysis: IL28B and ISDR.

DISCUSSION

The present study investigated 977 patients with genotype 1 using direct sequencing of core and NS5A regions, revealing that genotype 1a is rare (3.3%) in

Japan. Of the 33 patients with genotype 1a, 23 (71.9%) were patients with hemophilia, confirming that the majority of cases with genotype 1a involve patients with hemophilia who have received imported clotting factors, as previously reported [Fujimura et al., 1996; Otagiri et al., 2002; Hayashi et al., 2003]. Analysis after excluding patients with hemophilia revealed the prevalence of genotype 1a in Japan was 0.9% (9/954). Recently, the distributions of HBV genotypes have been changing in Japan due to international exchange [Hayashi et al., 2007; Matsuura et al., 2009]. However, prevalences of HCV genotypes have remained stable because of the different modes of infection involved. The present study revealed that 11 (47.8%) of 23 patients achieved sustained virological response. The IFN responsiveness of HCV genotype 1a in Japanese patients was reported in 1999 from Okinawa, a far southern island in Japan [Sakugawa et al., 1997]. That study reported that the rate of sustained virological response tended to be higher in patients with genotype 1a than in those with genotype 1b, but no significant differences were identified because of the small number of patients with genotype 1a. Low virological response rates in both genotypes 1a and 1b were confirmed in the present Japanese patients, as in Caucasian patients [Manns et al., 2001; McHutchison et al., 2009]. No significant differences in sustained virological response rate were seen between genotypes 1a and 1b. Discriminating between genotypes 1a and 1b thus seems to have little clinical relevance in terms of IFN responsiveness. Viral factors associated with sustained virological response, including HCV genotype, have been studied most frequently and mutations in the core and NS5A regions of HCV genotype 1b have been associated with response to IFN therapy [Akuta et al., 2005, 2010, 2011; Okanoue et al., 2009; Nakagawa et al., 2010; Toyoda et al., 2010; Hayashi et al., 2011a; Hayes et al., 2011; Kumthip et al., 2011; Kurosaki et al., 2011]. These viral factors could improve prediction of sustained virological response for genotype 1a, as in 1b. Amino acid substitutions at positions 70 and 91 of the HCV core region in genotype 1b have been related to IFN responsiveness, liver steatosis, hepatic oxidative stress, insulin resistance, and carcinogenesis [Akuta et al., 2005, 2007, 2009; Tachi et al., 2010]. These substitutions may have substantial impacts on

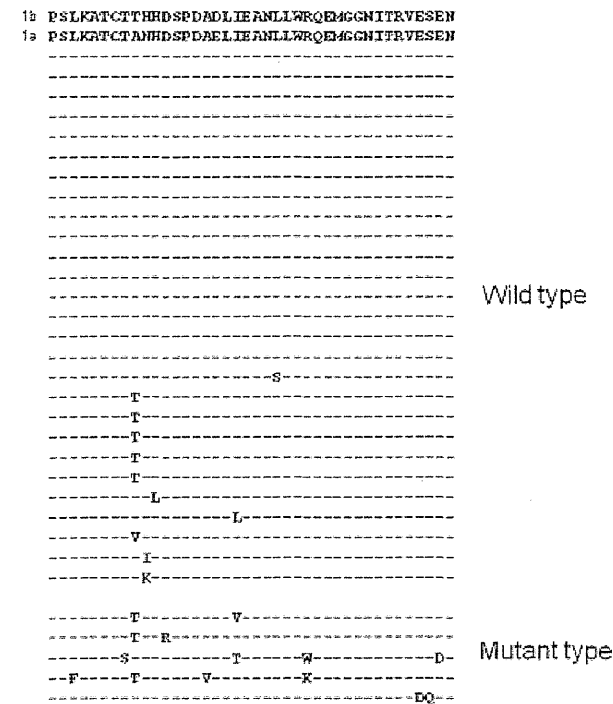


Fig. 1. Alignment of the amino acid sequence for the NS5A-ISDR. In the sequence alignment, dashes indicate amino acids identical to consensus sequence HCV1. Sequences of the HCV1 strain and HCV1 strains with one-nucleotide substitutions were defined as wild-type ISDR, and all other strains were defined as mutant-type ISDR. ISDR, interferon sensitivity-determining region.

TABLE IV. Univariate Analysis: Factors Predictive of Sustained Virologic Response

Factors	Sustained virologic response (n = 11)	Non-sustained virologic response (n = 12)	P-value
Age (y.o.)	37.9 ± 10.9	39.8 ± 11.3	0.6958
Gender: male/female	10/1	10/2	0.9999
ALT (IU/L)	78.2 ± 50.8	62.6 ± 68.1	0.5435
AST (IU/L)	51.4.4 ± 29.2	48.8 ± 40.4	0.8616
PLT (×10 ³ /mm ³)	19.0 ± 5.4	19.3 ± 5.7	0.8870
HCV RNA level (KIU/ml)	1323.1 ± 1077.3	2567.0 ± 2940.8	0.2481
ISDR: wild/mutant	7/4	12/0	0.0373
IL28B:TT/TG	9/1	4/8	0.0115

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; IL28B, interleukin 28B.

the pathogenesis of HCV genotype 1a infection. However, the HCV core region of genotype 1a is well-conserved and no significant mutations were seen in the core region, which is associated with IFN responsiveness. Several reports have also found that the HCV core region, including positions 70 and 91, of HCV genotype 1a is highly conserved [Alestig et al., 2011; Kumthip et al., 2011]. Mutations in the core region of genotype 1a would be rare, so this region might be unsuitable for routine clinical use, unlike in genotype 1b. However, the number of patients in this study was small, and large studies including from other countries are needed to clarify these issues. The ISDR in the NS5A region of HCV genotype 1b is closely associated with response to IFN therapy. ISDR mutations of genotype 1b are well known to be more important in predicting sustained virological response in Japanese patients than European patients [Hofgärtner et al., 1997; Zeuzem et al., 1997; Nakano et al., 1999; Pascu et al., 2004; Hayashi et al., 2011a]. European studies have failed to detect the specific amino acid substitutions in ISDR of genotype 1a associated with IFN responsiveness [Hofgärtner et al., 1997; Zeuzem et al., 1997]. In this study, sustained virological response was achieved in 36.8% of patients with wild-type ISDR and 100% of patients with mutant-type ($P = 0.0373$). The present analysis showed a close relationship between ISDR of genotype 1a and sustained virological response, as in genotype 1b. Recent investigations in Thailand and Iran have failed to identify the usefulness of ISDR for HCV genotype 1a in predicting sustained virological response [Kumthip et al., 2011; Yahoo et al., 2011]. The high virological response rate and low prevalence of patients with mutations in the ISDR do not favor the use of ISDR analysis in predicting IFN responsiveness [Herion and Hoofnagle, 1997; Yokozaki et al., 2011]. Rates of sustained virological response among these studies were much higher than those in the present study (68.4% and 75% vs. 47.8%). The mean number of mutations in patients who achieved sustained virological response in the studies by Kumthip et al. [2011] and Yahoo et al. [2011], and the present group were 1.4, 1.4, and 1.6, respectively. Differences in sustained virological response and the number of mutations to the ISDR might underpin this discrepancy in the evaluation of ISDR. Although the sample size in

the present study was small, the results indicate that ISDR represents a strong indicator of progression to sustained virological response for patients with HCV genotype 1a. Amino acid substitutions in the ISDR of genotype 1a thus also play an important role in predicting sustained virological response in Japanese patients compared to patients from other countries. IL28B polymorphisms such as host genetics, as well as mutations in the HCV genome, contribute to IFN treatment outcomes. Rates of sustained virological response in patients in this study with TT and TG were 69.2% and 11.1%, respectively. The TG allele of the IL28B genotype was significantly associated with poor response to IFN therapy ($P = 0.0115$). SNPs of IL28B would regulate the expression of IFN-stimulated genes and affect IFN responsiveness. IL28B and ISDR thus exert independent effects on IFN responsiveness and both host and viral factors impacting IFN responsiveness would improve the prediction of sustained virological response. Several studies have thus reported that both the SNP of IL28B and mutations in the ISDR were associated with sustained virological response in patients with HCV genotype 1b [Akuta et al., 2011; Hayashi et al., 2011b; Kurosaki et al., 2011]. In the present study of HCV genotype 1a, among the 9 patients who had simultaneously the TG allele for IL28B and wild-type ISDR, only 1 achieved sustained virological response (11.1%). The best-sustained virological response was achieved in patients with mutant-type ISDR and the T allele (100%). The combination of SNPs for IL28B and mutations in ISDR may thus predict response to IFN therapy in patients with HCV genotype 1a as well as genotype 1b. Given the small sample size in this investigation, larger cohorts are needed to confirm the present results. Furthermore, infection with genotype 1a in Japanese patients is rare, making large-scale studies difficult to perform.

In conclusion, the prevalence of HCV genotype 1a is rare in Japan and the majority of cases involve patients with hemophilia. The TG genotype of IL28B is associated with poor response, while mutant-type ISDR is associated with good response to combination therapy with pegylated-IFN- α 2b and ribavirin in patients with HCV genotype 1a. Combined use of both IL28B and ISDR could improve the prediction of IFN response.

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Efficacy and safety of eltrombopag in Japanese patients with chronic liver disease and thrombocytopenia: a randomized, open-label, phase II study

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Abstract

Background Eltrombopag is an oral thrombopoietin receptor agonist that stimulates thrombopoiesis and shows higher exposure in East Asian patients than in non-Asian patients. We evaluated the pharmacokinetics, efficacy, and safety of eltrombopag in Japanese patients with thrombocytopenia associated with chronic liver disease (CLD).

Methods Thirty-eight patients with CLD and thrombocytopenia (platelets <50,000/ μ L) were enrolled in this phase II, open-label, dose-ranging study that consisted of 2

parts. In the first part, 12 patients received 12.5 mg of eltrombopag once daily for 2 weeks. After the evaluation of safety, 26 patients were randomly assigned to receive either 25 or 37.5 mg of eltrombopag once daily for 2 weeks in the second part.

Results Pharmacokinetics showed that the geometric means of the maximum plasma concentration (C_{max}) and the area under the curve (AUC) in the 12.5 mg group were 3,413 ng/mL and 65,236 ng h/mL, respectively. At week 2, the mean increases from baseline in platelet counts were

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24,800, 54,000, and 60,000/ μ L in the 12.5, 25, and 37.5 mg groups, respectively. The median platelet counts increased within 2 weeks of the beginning of administration in all groups, and remained at the same level throughout the 2-week post-treatment period in the 12.5 mg group, whereas the platelet counts peaked a week after the last treatment in both the 25 and 37.5 mg groups. Most adverse events reported were grade 1 or 2; 2 patients in the 37.5 mg group had drug-related serious adverse events.

Conclusions Eltrombopag ameliorated thrombocytopenia in Japanese patients with CLD and thrombocytopenia. The recommended dose for these patients is 25 mg daily for 2 weeks.

Keywords Thrombopoietin receptor agonist · Pharmacokinetics · Invasive procedures · Inter-ethnic difference

Introduction

Thrombocytopenia is frequently observed in patients with chronic liver disease (CLD) and is considered a surrogate marker for the severity of liver disease [1, 2]. Besides hypersplenism secondary to portal hypertension, decreased thrombopoietin (TPO) production by hepatocytes is an important cause of thrombocytopenia in this patient population [3].

Some invasive procedures, such as liver biopsy, radiofrequency ablation (RFA), and partial hepatectomy for hepatocellular carcinoma (HCC), are performed as part of the therapeutic management of patients with CLD. Thrombocytopenia often interferes with such invasive procedures and platelet transfusions may be required [4–9]. The frequency of splenectomy and platelet transfusions is significantly higher in HCC patients with severe thrombocytopenia (<50,000/ μ L) than in those without thrombocytopenia [10]. However, both splenectomy and platelet transfusions have limitations and disadvantages; for example, splenectomy is invasive and may be associated with life-threatening short- as well as long-term complications, and platelet transfusions are short-acting and may cause transfusion-related complications [11–14]. Thus, alternative therapeutic options to platelet transfusions or splenectomy would provide an important clinical benefit.

Eltrombopag (GlaxoSmithKline, Ware, UK) is an orally bioavailable, small-molecule, non-peptide thrombopoietin receptor (TPO-R) agonist, which has been approved for the treatment of chronic idiopathic thrombocytopenic purpura (ITP). Eltrombopag induces the proliferation and differentiation of megakaryocytes, resulting in an increase in

platelet production in chimpanzees and humans [15]. Eltrombopag increases platelet counts in a dose-dependent fashion in patients with ITP and in those with thrombocytopenia with hepatitis C virus (HCV) infection, as well as in healthy volunteers [16–19].

Eltrombopag is primarily metabolized in the liver, and a higher plasma eltrombopag exposure has been reported in HCV-infected patients compared with ITP patients and healthy volunteers [20]. Furthermore, inter-ethnic differences in the pharmacokinetics of eltrombopag have been reported; the area under the curve (AUC) of eltrombopag in ITP patients and healthy volunteers was approximately 2-fold higher in East Asian subjects than in those of non-Asian origin [21]. Thus, the pharmacokinetics of eltrombopag in Japanese patients with CLD may be different from those previously reported in ITP patients and Caucasian patients [17–19].

The aim of this phase II study was to assess the efficacy and safety of eltrombopag in Japanese patients with CLD and thrombocytopenia using lower daily doses (12.5, 25, or 37.5 mg) than those typically used for Caucasian patients.

Methods

Patients

A total of 38 patients with CLD (25 with HCV infection, 7 with hepatitis B virus [HBV] infection, 1 with both HCV and HBV infections, and 5 with cryptogenic cirrhosis) were enrolled from 10 Japanese institutions between January and August 2009. Eligible patients were 20 years of age or older and had thrombocytopenia (baseline platelet counts <50,000/ μ L). Patients were also required to have a Child–Pugh score of 9 or less (Child–Pugh class A or B) and hemoglobin concentration of >8 g/dL for at least 4 weeks before enrollment. Platelet transfusions and interferon therapies had to be completed at least 2 and 4 weeks before enrollment, respectively.

Patients with evidence of human immunodeficiency virus (HIV) infection, evidence of portal vein thrombosis on abdominal imaging within 3 months before enrollment, a history of arterial or venous thrombosis with 2 or more thrombosis risk factors, or platelet agglutination abnormalities were excluded from the study. Patients with active World Health Organization (WHO) grade 3 or 4 bleeding were also excluded [22]. Women who were pregnant or breastfeeding were not eligible, nor were patients who required the use of polyvalent cation-containing medicines, which are known to form chelates with eltrombopag. Patients requiring medications that are known to affect platelet functions [e.g., aspirin, nonsteroidal

anti-inflammatory drugs (NSAIDs), and anti-platelet agents], and patients requiring hydroxymethylglutaryl-CoA reductase inhibitors (for which exposure might be increased by eltrombopag administration) were also excluded.

Diagnosis of liver cirrhosis was assessed by an aspartate aminotransferase-to-platelet ratio index (APRI) of >1 [23] and an FIB4 index of >3.25 according to the Practice Guideline for Liver Cirrhosis edited by the Japanese Society of Gastroenterology [24]. Creatinine clearance was estimated by the Cockcroft–Gault formula [25] in a post-hoc analysis.

This study was approved by each institutional review board and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and local laws and regulations. All patients provided written informed consent before enrollment.

Treatments

Because inter-ethnic differences in the eltrombopag AUC have been found in earlier studies [21, 26], lower doses (12.5, 25, 37.5 mg) of eltrombopag were used than the doses (30, 50, 75 mg) used in a previous study conducted in cirrhotic patients (predominantly Caucasian) with HCV infection [18]. All doses were administered with the patients in a fasting state, in which the patients were required to refrain from food ingestion for at least 2 h pre- and post-dose.

Study design and procedures

This was a multicenter, open-label, dose-ranging phase II study that used a unique sequential design and consisted of 2 parts. In the first part, 12 patients received 12.5 mg of eltrombopag once daily for 2 weeks, and the data were reviewed by a Safety Review Committee (Fig. 1). After the evaluation of safety in the first part, in the second part 26 new patients were randomly allocated, at a 1:1 ratio, to receive either 25 or 37.5 mg of eltrombopag once daily for 2 weeks. An additional week of treatment was allowed if platelet counts were $<80,000/\mu\text{L}$ at week 2 (Fig. 1). Eltrombopag treatment was to be discontinued if platelet counts were $>200,000/\mu\text{L}$ during the treatment period. Invasive procedures could be performed after the end of treatment with eltrombopag per the investigator's decision. All patients were assessed for the efficacy and safety of eltrombopag every week during the treatment period, and at 4 days, 1 week, and 2 weeks post-treatment.

The primary endpoint of the study was the change from baseline in platelet counts at the end of week 2. Secondary endpoints were: (1) response rate (achieving platelet counts of $\geq 80,000/\mu\text{L}$) of eltrombopag administered for 2 weeks, or after an additional week, (2) median platelet counts, and (3) safety. The severity of adverse events was graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 1.9, dated December 2004). The effects of pretreatment with

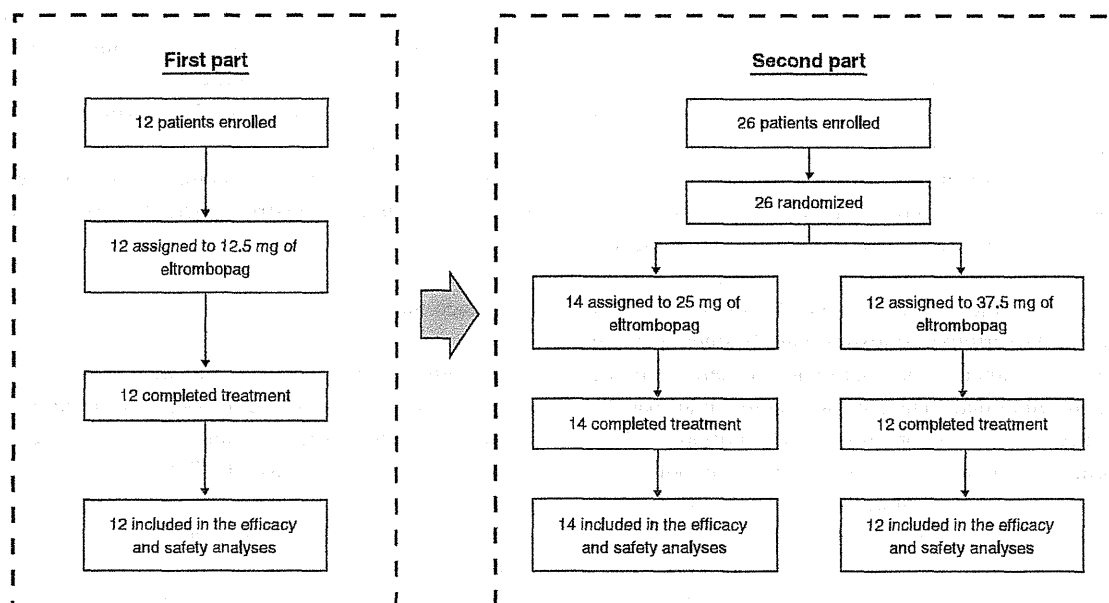


Fig. 1 Study design. The study was a multicenter, open-label, dose-ranging phase II study that used a unique sequential design and consisted of 2 parts. After review, by a Safety Review Committee, of

safety data from the 12.5 mg group (first part), new patients were randomly assigned to receive 25 or 37.5 mg of eltrombopag once daily for 2 weeks in the second part

eltrombopag on the prevalence of perioperative bleeding and platelet transfusions were also evaluated when invasive procedures, such as liver biopsy, RFA, and partial hepatectomy, were performed after the end of treatment.

Randomization and masking

In the second part of this study, patients were randomly allocated to either the 25 or 37.5 mg group. Randomization was centrally performed, and the random assignment was stratified according to baseline Child–Pugh class (A or B). This study was not blinded.

Pharmacokinetics

Serial samples were collected pre-dose (prior to administration on day 14), and 1, 2, 4, 6, 8, 10, and 24 h post-dose in the 12.5 mg group. Pharmacokinetic parameters [maximum plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}), AUC_{0-t} , and $AUC_{0-\infty}$] in the 12.5 mg group were determined with actual sample times using non-compartmental analysis and summary statistics. Sparse samples were collected in the 25 or 37.5 mg group following 1 of 2 schedules: (1) pre-dose (prior to administration on day 14) and 0.5–3, and 24 h post-dose, or (2) 4–6, 8–12, and 24 h post-dose. The geometric mean of the plasma eltrombopag concentration was summarized in each group. One patient each from the 12.5 and 37.5 mg groups was excluded from the summary statistics, as these 2 patients had used a cation-containing antacid.

Statistical analyses

On the basis of a previous study [18], the changes from baseline in platelet counts at week 2 were assumed to be 30,000, 80,000, and 100,000/ μ L in the 12.5, 25, and 37.5 mg groups, respectively, and the standard deviation was 50,000/ μ L in each group. Based on Monte Carlo simulations, 12 evaluable patients per group were needed to provide 90 % or more power to detect a linear dose trend and saturation at a medium dose trend. No interim analysis was planned. Descriptive statistics and frequency tables were used to summarize demographics, baseline characteristics, and safety data. The analyses included all patients who had received at least one dose of eltrombopag.

The primary endpoint was analyzed using point estimates and 2-sided 95 % confidence intervals (CIs) by each group. In an exploratory analysis, the changes from baseline in platelet counts at week 2 were analyzed using analysis of covariance (ANCOVA), with baseline platelet counts as a covariate to detect the following dose response patterns: linearity in 3 doses, saturation at the medium dose (25 mg), or onset of response at the high dose (37.5 mg).

A similar analysis, with both baseline platelet counts and Child–Pugh class as covariates, was conducted as a secondary analysis. This model used the changes in platelet counts of each patient. No adjustment for multiplicity was made because these analyses were exploratory. Other secondary endpoints were analyzed using point estimates and 2-sided 95 % CIs for each group. Analyses were based on the observed data.

This study is registered at ClinicalTrials.gov with identifier number: NCT00861601.

Role of the funding source

The protocol was developed by the principal investigators and employees of the sponsor. Data were collected and analyzed by the sponsor. All authors had access to the primary data and vouch for the completeness and accuracy of the data and analyses. Interpretation of the data and decisions related to the content of the report were made through collaboration among all authors. The corresponding author had final responsibility for the decision to submit for publication.

Results

Patient characteristics

Patients' characteristics are summarized in Table 1. There were no marked differences in age, sex, body mass index (BMI), Child–Pugh classification, or creatinine clearance among the groups. In addition, no apparent differences were seen in baseline platelet counts among the groups. All the enrolled patients showed APRI >1 and/or FIB4 >3.25 in a post-hoc analysis. Furthermore, 87 % of the enrolled patients (33/38) had 1 of the following complications of liver cirrhosis: edema (2/38), ascites (6/38), esophageal and/or gastric varices (26/38), or HCC (18/38).

Pharmacokinetics

The geometric mean of C_{max} in the 12.5 mg group was 3,413 ng/mL (95 % CI 2,549–4,570) at approximately 3.4 h after administration, and the geometric mean of the AUC (0–24) was 65,236 ng h/mL (95 % CI 46,748–91,035) (Table 2). There was no apparent difference in the mean plasma eltrombopag concentration stratified by Child–Pugh class in the 12.5 and 25 mg groups (Fig. 2a, b). However, in the 37.5 mg group a higher mean plasma concentration of eltrombopag was observed in patients with Child–Pugh class B compared with patients with Child–Pugh class A (Fig. 2c).

Table 1 Patient characteristics

		12.5 mg (N = 12)	25 mg (N = 14)	37.5 mg (N = 12)
Age (years)	Median (range)	63.0 (45–81)	58.0 (44–75)	69.5 (48–81)
Sex	Female/male	4/8	4/10	4/8
Body mass index (kg/m ²)	Mean ± SD	22.6 ± 2.20	25.0 ± 4.15	24.7 ± 4.72
Etiology of liver disease	HCV/HBV/cryptogenic	7/4/1	9/3/2	10/1/2 ^a
Child–Pugh classification	A/B	8/4	8/6	7/5
APRI	Mean ± SD	4.3 ± 2.0	4.9 ± 2.8	4.9 ± 2.8
FIB4	Mean ± SD	12.7 ± 3.6	13.8 ± 4.5	16.5 ± 8.2
Baseline platelet count (/μL)	Median (range)	42,500 (36,000–49,000)	38,000 (19,000–48,000)	40,000 (23,000–49,000)
Total bilirubin (mg/dL)	Mean ± SD	1.51 ± 1.19	1.53 ± 0.62	1.27 ± 0.52
Creatinine (mg/dL)	Mean ± SD	0.70 ± 0.22	0.72 ± 0.16	0.83 ± 0.26
Creatinine clearance (mL/min)	Mean ± SD	93.5 ± 29.7	106.1 ± 34.9	84.2 ± 40.4

HBV hepatitis B virus, HCV hepatitis C virus, SD standard deviation, APRI aspartate aminotransferase-to-platelet ratio index

^a One patient in the 37.5 mg group was infected with both HCV and HBV

Table 2 Pharmacokinetic parameters (12.5 mg eltrombopag group, log-transformed data)

	N	n ^a	Geom. mean	95 % CI of geom. mean		SD logs	%CVb
				Lower	Upper		
C _{max} (ng/mL)	12	11	3,413	2,549	4,570	0.4345	45.6
T _{max} (h)	12	11	3.44	2.459	4.823	0.5012	53.4
AUC(0–t) (ng h/mL)	12	11	65,244	46,617	91,314	0.5004	53.3
AUC(0–24) (ng h/mL)	12	11	65,236	46,748	91,035	0.4960	52.8

CI confidence interval, Geom. mean geometric mean, CVb between-subject coefficient of variance, C_{max} maximum plasma concentration, T_{max} time to maximum plasma concentration

^a One patient was excluded from the summary statistics of pharmacokinetic parameters because the patient had used a cation-containing antacid, which affects the exposure of eltrombopag

Efficacy

Primary endpoint

Changes from baseline in platelet counts at week 2

The mean increases from baseline in platelet counts at week 2 were 24,800/μL (95 % CI 8,200–41,400), 54,000/μL (95 % CI 28,200–79,800), and 60,000/μL (95 % CI 29,300–90,700) in the 12.5, 25, and 37.5 mg groups, respectively (Fig. 3). An exploratory analysis showed statistically significant linearity in 3 doses ($p = 0.0104$) and saturation at the medium dose ($p = 0.0057$) at the 5 % significance level.

Secondary endpoints

Response rate to eltrombopag and effects of an additional 1-week treatment

There were 3 (25 %), 6 (42.9 %), and 7 (58.3 %) patients in the 12.5, 25, and 37.5 mg groups, respectively, who responded (achieved platelet counts of $\geq 80,000/\mu\text{L}$) to eltrombopag at week 2. Six patients in the

25 mg group and 2 patients in the 37.5 mg group with platelet counts of $< 80,000/\mu\text{L}$ at week 2 received an additional 1 week of treatment. Of these patients, 3 in the 25 mg group and 1 in the 37.5 mg group responded to the additional week of treatment. Platelet counts in these 4 patients increased to approximately 70,000/μL (range 69,000–74,000) by week 2.

Median platelet counts after administration of eltrombopag The median platelet count in the 12.5 mg group increased from 42,500/μL [inter-quartile range (IQR) 40,500–45,500] at baseline to 66,000/μL (IQR, 45,000–83,000) at week 2 and remained at the same level for 2 weeks post-treatment. In contrast, in the 25 and 37.5 mg groups, the median platelet counts increased to 73,000/μL (IQR, 69,000–110,000) and 81,500/μL (IQR, 69,500–114,000), respectively, by week 2. At 1-week post-treatment, the median platelet counts peaked at 119,000/μL (IQR, 90,000–141,000) and 120,000/μL (IQR, 95,500–175,500) in the 25 and 37.5 mg groups, respectively, and remained at $> 80,000/\mu\text{L}$ for 1 week thereafter (Fig. 4). The median increases from baseline in platelet counts at week 2 for

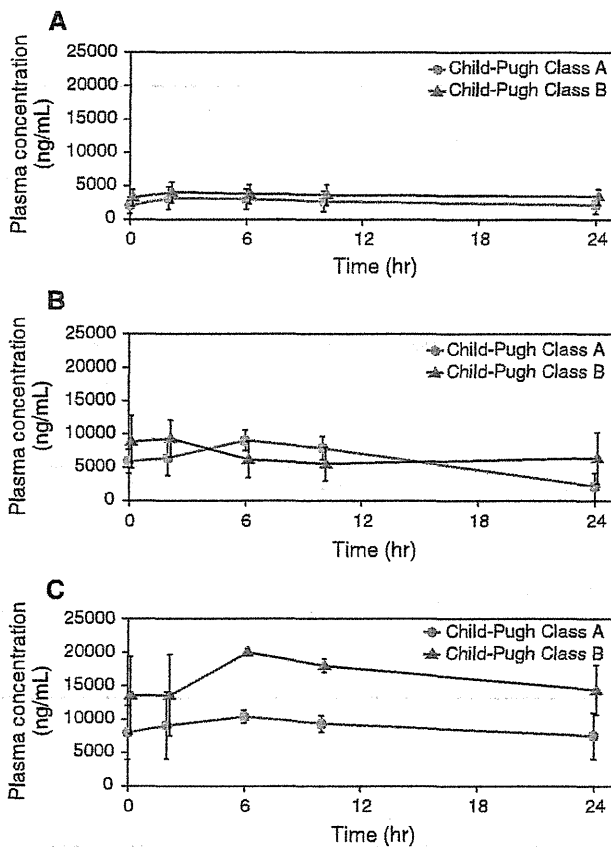


Fig. 2 Plasma eltrombopag concentration stratified by Child–Pugh class in the 12.5 mg (a), 25 mg (b), and 37.5 mg groups (c). One patient in the 12.5 mg group with Child–Pugh class A and another patient in the 37.5 mg group with Child–Pugh class B were excluded from summary statistics of plasma concentration, because both patients had used a cation-containing antacid, which affects the exposure of eltrombopag. Data are expressed as means ± SD

Child–Pugh class A and B, respectively, were 11,000/μL (range, –9,000 to 83,000) and 28,000 (range, 17,000–30,000) in the 12.5 mg group; 38,500/μL (range, 12,000–100,000) and 50,000 (range, 19,000–187,000) in the 25 mg group; and 46,000/μL (range, 8,000–193,000) and 50,000 (range, 20,000–91,000) in the 37.5 mg group.

Safety

The incidences of adverse events (AEs) of any grade during the study were 50 % (6/12), 50 % (7/14), and 75 % (9/12) in the 12.5, 25, and 37.5 mg groups, respectively (Table 3). Most AEs reported were grade 1 or 2 in severity. Back pain, pyrexia, and postoperative fever were the most common AEs, and they occurred mostly after invasive procedures. No grade 3 or higher AEs occurred during the treatment period. No subject discontinued eltrombopag because of AEs or platelet counts of >200,000/μL during the study.

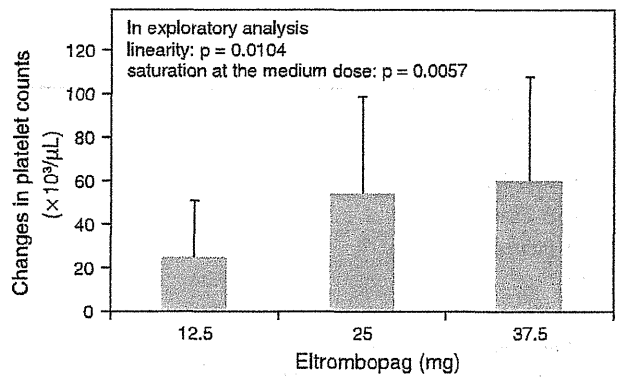


Fig. 3 Changes from baseline in platelet counts at week 2. Exploratory analyses were conducted to detect a dose response and trend, using the changes from baseline in platelet counts at week 2. These data were analyzed using analysis of covariance (ANCOVA) with baseline platelet counts as a covariate, using contrast methods for the following dose response patterns: linearity in 3 doses [contrast of 12.5, 25 and 37.5 mg: –1 0 1], saturation at the medium dose (25 mg) [contrast: –2 1 1], and onset of response at the high dose (37.5 mg) [contrast: –1 –1 2]. No adjustment for multiplicity was made. Data are expressed as means + SD

Drug-related AEs occurred in 8 % (1/12), 29 % (4/14), and 33 % (4/12) of patients in the 12.5, 25, and 37.5 mg groups, respectively (Table 3). No drug-related serious adverse events (SAEs) were seen in either the 12.5 or 25 mg groups; however, 2 patients in the 37.5 mg group experienced drug-related SAEs (Table 3).

Drug-related SAEs

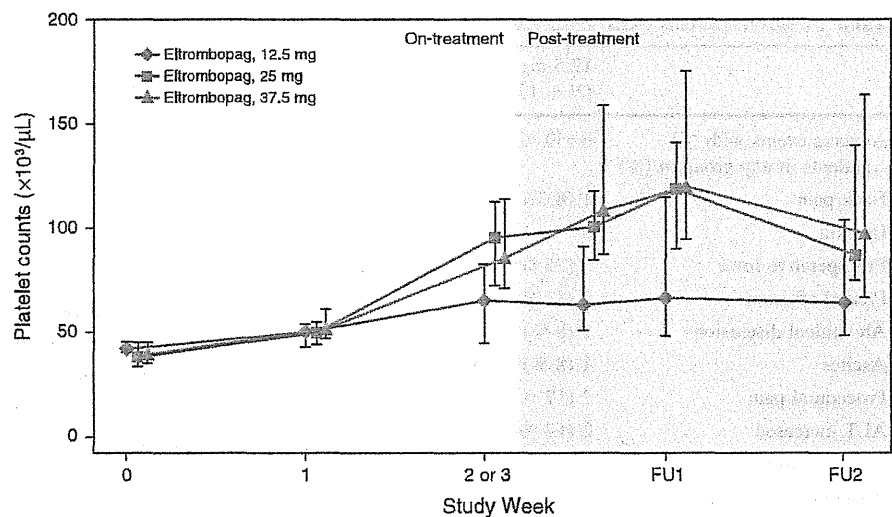
SAE#1; worsening pleural effusion and development of portal vein thrombosis

A 63-year-old female cirrhotic patient with HCC, esophageal varices, and pleural effusion was administered 37.5 mg of eltrombopag daily for 14 days. Her platelet count increased from 36,000 to 127,000/μL and there were no AEs during eltrombopag administration. On day 23, the patient underwent partial splenic embolization. On day 35, grade 3 worsening pleural effusion and grade 3 portal vein thrombosis were seen. Platelet counts were 197,000 and 271,000/μL on days 22 and 35, respectively. With conservative therapy, the pleural effusion and portal vein thrombosis improved, on days 77 and 140, respectively.

SAE#2; worsening ascites

An 81-year-old female cirrhotic patient with HCC and ascites was administered 37.5 mg of eltrombopag daily for 14 days. Her platelet count increased from 49,000 to 242,000/μL. Although no thrombus was observed on computed tomography (CT) images, grade 2 worsening ascites was seen on day 11. The ascites was refractory to

Fig. 4 Median platelet counts after treatment with eltrombopag. Platelet counts at either week 2 or 3, or at the end of treatment with eltrombopag. Platelet counts after the end of treatment include the values after invasive procedures or platelet transfusions. Data are expressed as medians with interquartile ranges (IQRs). FU follow up



diuretic agents and an albumin preparation and was found to be chylous on day 57. Platelet counts were 87,000 and 197,000/ μL on days 9 and 57, respectively. The patient developed cachexia and renal failure, and died on day 163 (149 days after the end of eltrombopag treatment).

Effects of pretreatment with eltrombopag on the prevalence of perioperative bleeding and platelet transfusions

Of the 38 patients who received eltrombopag, 6 patients underwent a total of 7 invasive procedures with bleeding risk after the end of treatment with eltrombopag. RFA was the most common procedure during the study (Table 4). Five of these patients had platelet counts of $>80,000/\mu\text{L}$ prior to undergoing their invasive procedures, and most of the procedures were safely performed without platelet transfusions (Table 4).

Discussion

This study demonstrated that significant increases in platelet counts could be achieved by 2-week administration of eltrombopag to Japanese patients with CLD and thrombocytopenia. Our results also show that a maximum of 25 mg of eltrombopag, a lower dose than that typically used in Caucasian patients, can be recommended for Japanese patients with CLD and thrombocytopenia.

In this phase II study, we investigated the pharmacokinetics of eltrombopag in Japanese patients with CLD and thrombocytopenia. An inter-ethnic difference in the pharmacokinetics of eltrombopag has been observed between East Asian and non-Asian patients with ITP, as well as between East Asian and non-Asian healthy volunteers [21];

in our study, the $\text{AUC}_{0-\tau}$ in Japanese patients receiving 37.5 mg of eltrombopag once daily was estimated to be 236 $\mu\text{g h/mL}$, which is similar to that seen in non-East Asian patients with CLD receiving 75 mg once daily in a previous study [26]. Although the mechanisms underlying an inter-ethnic difference in the pharmacokinetics of eltrombopag remain unclear, a common difference between East Asian and Caucasian ethnic groups is body weight [27]. Because the clearance of eltrombopag increased with body weight [21] and because the body weight of East Asian patients is lower than that of Caucasian patients in general, body weight differences could account for the differences in serum levels of eltrombopag seen between the two groups. A pharmacogenetic study has also been performed to investigate a relationship between gene polymorphisms and inter-ethnic differences; however, the responsible polymorphism has not been identified (data not shown). Eltrombopag is a substrate of several drug-metabolizing enzymes, including cytochrome P450 (CYP) 1A2, CYP2C8, uridine diphosphate-glucuronosyltransferase (UGT) 1A1, and UGT1A3, and the agent is also a substrate of breast cancer resistance protein [27]. Because the activities of these enzymes are known to have inter-ethnic differences [28, 29], it is possible that multiple factors, including genetic differences in metabolizing enzymes and transporters, may be involved in the observed difference [27].

In the present study, platelet counts continued to increase 1 week post-treatment and gradually decreased thereafter. This finding is similar to that seen in another study of eltrombopag in patients with CLD and thrombocytopenia; in contrast, in several studies of eltrombopag in chronic ITP, platelet counts began to decrease at 1 week post-treatment and returned to baseline levels within 2 weeks [19, 30]. Although the reason is unclear, 2

Table 3 Adverse events (AEs) observed during the study

	12.5 mg (N = 12)	25 mg (N = 14)	37.5 mg (N = 12)
Adverse events with ≥ 2 patients in any group, n (%)	6 (50 %)	7 (50 %)	9 (75 %)
Back pain	1 (8 %)	0	4 (33 %)
Pyrexia	0	3 (21 %)	2 (17 %)
Postoperative fever	3 (25 %)	0	2 (17 %)
Pleural effusion	2 (17 %)	0	2 (17 %)
Abdominal distension	1 (8 %)	0	2 (17 %)
Ascites	1 (8 %)	0	2 (17 %)
Procedural pain	2 (17 %)	0	1 (8 %)
ALT increased	2 (17 %)	1 (7 %)	0
AST increased	2 (17 %)	1 (7 %)	0
All grade 3 or 4 adverse events, n (%)	0	0	1 (8 %)
All drug-related adverse events, n (%)	1 (8 %)	4 (29 %)	4 (33 %)
Diarrhea	0	1 (7 %)	1 (8 %)
Renal impairment	0	1 (7 %)	1 (8 %)
Abdominal distension	0	0	1 (8 %)
Abdominal pain	0	0	1 (8 %)
Back pain	0	0	1 (8 %)
Eosinophilia	0	0	1 (8 %)
Eosinophil count increased	0	0	1 (8 %)
Anorexia	0	0	1 (8 %)
Pleural effusion	0	0	1 (8 %)
Pain in extremity	0	1 (7 %)	0
Vomiting	0	1 (7 %)	0
Urinary tract infection	0	1 (7 %)	0
Supraventricular extrasystoles	1 (8 %)	0	0
Serious adverse events, n (%)	0	0	2 (17 %)
Ascites	0	0	1 (8 %)
Pleural effusion	0	0	1 ^a (8 %)
Portal vein thrombosis	0	0	1 ^a (8 %)
Death, n (%)	0	0	1 ^b (8 %)

The severity of adverse events was graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 1.9, dated December 2004). The data include AEs seen on study plus all drug-related AEs

ALT alanine aminotransferase, AST aspartate aminotransferase

^a One patient experienced pleural effusion and portal vein thrombosis 22 days post-treatment

^b The death occurred 149 days after the end of treatment with eltrombopag

possibilities may account for this difference. First, plasma eltrombopag concentrations in patients with CLD are higher than those in patients with ITP [21, 26], and therefore the differences in exposure between these 2 diseases may be responsible for changes in platelet counts after treatment with eltrombopag. Second, the difference

may be based on the pathogenesis of thrombocytopenia. The main cause of thrombocytopenia in patients with ITP is an autoimmune-mediated active platelet destruction. In patients with CLD, increased blood flow into the spleen secondary to portal hypertension and subsequent passive trapping of platelets in the spleen contribute to thrombocytopenia in this patient population [31, 32].

Most AEs reported in the present study were grade 1 or 2 in severity, and no significant aminotransferase abnormalities were observed. The incidence of drug-related AEs in the 37.5 mg group was somewhat higher compared with the other groups, and drug-related serious events (ascites, increase of pleural effusion, and development of portal vein thrombosis) were seen in 2 patients receiving 37.5 mg of eltrombopag. There was 1 portal vein thrombosis event seen after eltrombopag treatment. Post-hoc analyses of a study of non-Japanese patients with CLD and thrombocytopenia receiving 75 mg of eltrombopag showed that maximum post-baseline platelet counts were associated with the thromboses observed in that study [33]. Thus, thrombogenesis seems to be a factor for the development of the thrombotic events. In addition, it has recently been reported that platelets can amplify inflammation [34], suggesting that platelet-amplified inflammation could be a possible factor for the development of thrombosis. The minimum effective dose of eltrombopag should therefore be used in order to minimize the risk of thromboembolic events, and platelet counts should be closely monitored.

It may be unusual to recommend an optimal dose of eltrombopag in Japanese patients with CLD based only on this study. Nevertheless, we propose that 12.5 or 25 mg can be recommended for Japanese patients with CLD and thrombocytopenia undergoing invasive procedures, based on the following 3 findings: (1) a higher mean concentration of eltrombopag was observed in patients with Child–Pugh class B compared with Child–Pugh class A in the 37.5 mg group (Fig. 2); (2) although the mean $AUC_{0-\tau}$ increased with an increase in the eltrombopag dose, the increase in platelet counts seemed to be saturated at 25 mg of eltrombopag (Figs. 3, 4); (3) SAEs were seen in the 37.5 mg group. Although the SAEs may have been due in part to invasive procedures or the natural course of the disease, we could not rule out the possibility of these being drug-related.

In our study, except for 1 patient treated with partial hepatectomy, splenectomy, and cholecystectomy, perioperative bleeding was not seen and platelet transfusions were not required before invasive procedures. These findings suggested that eltrombopag may reduce the necessity for platelet transfusions in patients with CLD undergoing invasive procedures.

These findings demonstrated that a maximum daily dose of 25 mg of eltrombopag administered for 2 weeks was

Table 4 Effects of pretreatment with eltrombopag on the prevalence of perioperative bleeding and platelet transfusions

Groups	Case number	Procedure	Bleeding	Platelet transfusion	Days after end of treatment	Platelet count (μL)		
						Baseline	Pre-procedure	Post-procedure
12.5 mg	11	Partial hepatectomy, splenectomy, cholecystectomy	Yes	Yes ^a	8	42,000	46,000	194,000
	31	Radiofrequency ablation	No	No	3	43,000	173,000	178,000
25 mg	5	Radiofrequency ablation	No	No	13	48,000	217,000	152,000
	6	Tooth extraction	No	No	9	45,000	387,000	382,000
		Tooth extraction	No	No	13	45,000	387,000	382,000
	32	Liver biopsy	No	No	1	46,000	146,000	142,000
37.5 mg	51	Radiofrequency ablation	No	No	6	37,000	126,000	183,000

^a Platelet transfusion was performed prior to invasive procedures

effective and well-tolerated for Japanese patients with CLD. Eltrombopag seems to be an efficacious alternative to platelet transfusions for supporting invasive procedures in patients with CLD and thrombocytopenia, although there might be a risk of treatment-related thrombosis. Further studies will help to establish the appropriate use of eltrombopag for supporting invasive procedures in patients with CLD and thrombocytopenia while investigating the effective prevention of thrombosis.

In conclusion, eltrombopag ameliorated thrombocytopenia in Japanese patients with CLD. A daily dose of 25 mg and 2-week administration is recommended for these patients. Recently, Afdhal et al. [35] reported that patients with HCV who were treated with eltrombopag showed significantly higher sustained virologic response rates following interferon-based therapy compared with patients treated with placebo. Therefore, in addition to its role as a supporting agent for invasive procedures, further studies will be focused on the ability of eltrombopag to initiate and maintain the interferon therapy, and subsequently facilitate an increase in the sustained virologic response rate in patients with thrombocytopenia with chronic HCV infection.

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Conflict of interest Toshihiro Hattori and Koichi Katsura are employees of GlaxoSmithKline, Tokyo, Japan. All other authors declare no conflict of interest.

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