Table 1 Summary of investigations of acoustic radiation force impulse for assessment of liver fibrosis

Author (year) reference	Disease	Number of patients	System of fibrosis staging	Fibrosis stage							
				F> or =1		F> or =2		F> or =3		F> or =4	
				Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC	Cut-off value (m/s)	AUROC
Friedrich-Rust (2009)[10]	Chronic viral hepatitis	86	Metavir			1.37	0.82	1.45	0.91	1.75	0.91
Lupsor (2009)[23]	HCV	112	Metavir	1.19	0.725	1.34	0.869	1.61	0.9	2	0.936
Takahashi (2009)[21]	Chronic liver disease	55	Metavir			1.34	0.94	1.44	0.94	1.8	0.96
Fierbinteanu-Braticevici (2009)[22]	HCV	74	Metavir	1.185		1.215	0.902	1.54	0.993	1.94	0.993
Sporea (2011)[27]	Chronic liver disease	76	Metavir	1.4	0.747					1.78	0.951
Grgurevic (2011)[28]	Chronic liver disease	38	Ishak							1.86	0.99
Sporea (2010)[24]	Chronic viral hepatitis	71	Metavir			1.33	0.649			1.8	0.868
Toshima (2011)[25]	Chronic liver disease	79	Scheuer	1.45	0.81	1.52	0.81	1.69	0.85	1.79	0.87
Piscaglie (2011)[29]	Chronic liver disease	90								1.75	0.941
Ebinuma (2011)[26]	Chronic viral hepatitis	59	Metavir			1.4	0.905	1.53	0.923	1.88	0.854

AUROC, area under the receiver operating characteristic curve; HCV, hepatitis C virus.

specificity 80%), 17 of 42 patients with biopsy proven F2 (40%) had LS by TE corresponding to F0-1.[30] However, they are still useful for determining the indication of antiviral therapies, if we use them in combination with laboratory tests and other clinical data. The combination of TE and biomarkers is being studied to improve the diagnostic accuracy of significant fibrosis.[30,31]

PREDICTION OF RESPONSE TO ANTIVIRAL THERAPY

FIBROSIS STAGE IS an important predictor for response to combination therapy of pegylated interferon (PEG-IFN) and ribavirin for chronic hepatitis C. Hayashi *et al.* reported that the factors related to sustained virological response (SVR) on multivariate analysis were single nucleotide polymorphism (SNP) of interleukin 28B (IL28B) ($P = 0.0001$), fibrosis ($P = 0.0111$) and mutations in the core region70 ($P = 0.0267$) and IFN sensitivity determining region (ISDR) of HCV genome ($P = 0.0408$).[32]

Poynard *et al.* studied the predictive factors for SVR in 1459 patients with chronic hepatitis C retreated with PEG-IFN alfa-2b plus weight-based ribavirin. Uni- (UV) and multi-variable (MV) analyses were performed. Five baseline factors were associated ($P < 0.001$) with SVR in UV and MV analyses (odds ratio: UV/MV): fibrosis stage estimated using FibroTest (4.5/5.9) or biopsy (1.5/1.6), genotype 2/3 (4.5/5.1), viral load (1.5/1.3), prior relapse (1.6/1.6), previous treatment with non-PEG-IFN (2.6/2.0). Poynard *et al.* concluded that FibroTest at baseline is a possible non-invasive alternative to biopsy for the prediction of SVR, in patients with previous failures and advanced fibrosis, retreated with PEG-IFN alfa-2b and ribavirin.[33]

We have studied the predictive factors for SVR in 88 patients with chronic hepatitis C genotype 1 treated with combination of IFN and ribavirin and found that gender ($\beta = 1.6$, $P = 0.0012$) and LS by TE ($\beta = -0.1$, $P = 0.0214$) are independent predictive factors by multivariate analysis (manuscript in preparation).

Thus FibroTest and LS by TE can substitute liver biopsy for the purpose of predicting response to antiviral therapy in chronic hepatitis C.

EVALUATION OF EFFECTS OF ANTIVIRAL THERAPY

THE OUTCOME OF antiviral therapy should be assessed not only by ALT levels or viral loads but

also by the alleviation of fibrosis stage both in chronic hepatitis B and in chronic hepatitis C.

Ogawa *et al.* studied 145 HCV infected patients treated with PEG-IFN plus ribavirin by TE[34]. LS were significantly decreased in SVR patients (the mean rate of change; −16.2%, −32.2% and −43.5%) in comparison with non-SVR patients (−7.2%, −2.1% and +17.3%) at the end of treatment (EOT) ($P = 0.0127$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after EOT. Among non-SVR patients, LS were significantly decreased in patients with biochemical response (BR) (−17.9%, −30.0% and −27.1%) in comparison with non-BR (−4.1%, +6.4% and +30.6%) at EOT ($P = 0.0270$), and 48 weeks ($P < 0.0001$) and 96 weeks ($P < 0.0001$) after EOT.

Arima *et al.* measured LS by TE before treatment, at EOT, one year and 2 years after EOT in 145 patients with chronic hepatitis C treated by IFNs with or without ribavirin.[35] In 93 patients with SVR and 28 relapsers, LS significantly decreased at EOT (median, 5.4 [interquartile range, 4.0–8.6] kPa, $P < 0.0001$ and 6.8 [4.5–8.9] kPa, $P = 0.0023$) and one year after EOT (5.3 [4.2–7.0] kPa, $P < 0.0001$ and 6.8 [4.5–9.3] kPa, $P = 0.0204$) compared with baseline (8.0 [5.0–11.9] kPa and 10.6 [7.0–16.6] kPa). In SVR patients, LS significantly decreased 2 years after EOT (5.3 [4.1–6.3] kPa) compared with baseline ($P < 0.0001$) and LS at EOT ($P = 0.0034$). In 24 patients with non virological response (NVR), LS at EOT, one year after EOT, and 2 years after EOT did not significantly differ from pretreatment values.

Arima *et al.* proposed the use of deduced fibrosis stage from LS based on cut-off values for fibrosis stage. The use of deduced fibrosis stage enables evaluation of the degrees of changes of LS. 2-point or greater reduction of deduced stage was observed in 78% (29/37) of SVR patients, 59% (10/17) of relapsers and 15% (2/13) of NVR patients. A 2-point or greater decrease of deduced fibrosis stage were associated with milder baseline fibrosis stage, lower hyaluronic acid levels, longer IFN treatment, virological response of SVR or relapse and higher ALT levels.

Thus, we can assess not only the alleviation of fibrosis but also the factors that affect the alleviation of fibrosis by measuring LS in chronic hepatitis C.

Wang *et al.* studied LS by TE in 144 patients receiving IFN-based therapy, including 95 SVR patients and 49 non-SVR patients.[36] There was a significant decrease of LS among SVR patients (median, 0.6; $P < 0.001$). non-SVR patients showed an increase of LS (median, 0.8; $P = 0.557$). For SVR patients, a high initial LS was the predictive factor of a rapid reduction of LS values. However, advanced fibrosis stage before therapy, higher body mass index (BMI) and longer time remission were predictive factors for slow reduction of LS values.

Osakabe *et al.* measured LS by TE in 29 HBV-infected patients treated with nucleotide or nucleoside analogs and assessed the changes of LS.[37] By antiviral therapy, LS significantly reduced from 12.9 (6.2–17.9) kPa to 6.6 (4.4–10.3) kPa in the interval of 512 (366–728) days ($P < 0.0001$). Eleven of 19 (58%) patients with baseline fibrosis stages of F3-4 deduced from LS had 2-point or greater reduction of deduced stage at last LS measurement. The change ratio of hyaluronic acid ($P = 0.0390$) was associated with a 2-point or greater reduction.

Enomoto *et al.* studied LS by TE in 50 patients with chronic hepatitis B virus infection.[38] LS of the patients with entecavir significantly decreased from 11.2 kPa (7.0–15.2) to 7.8 kPa (5.1–11.9; $P = 0.0090$) during 12 months of treatment.

It is difficult to repeat liver biopsies after or during antiviral therapy to assess its effect. Since there is the heterogeneity of the effect of treatment, it is important to know who is a good responder or not and investigate the factors affecting the effect of therapy. Non-invasive measurement of LS can be done repeatedly and provide the information of effect of antiviral therapy.

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.

ASSESSMENT OF NATURAL COURSE OF VIRAL HEPATITIS

ARIMA *ET AL.* STUDIED 35 patients with chronic HCV infection without IFN treatment and reported that LS at 2nd measurement (12.2 [6.3–16.8] kPa) did not differ significantly from LS at 1st measurement (10.5 [5.8–15.3] kPa) in the interval of 656 (360–922) days.[35]

Osakabe *et al.* reported that, in 52 HBV-infected patients without antiviral therapy, LS tended to increase from 6.1 (3.9–8.5) kPa to 6.3 (4.4–9.7) kPa in the interval of 422 (358–709) days ($P = 0.0682$).[37] Without antiviral therapy, 11 of 50 (22%) patients with deduced fibrosis stages of F0-3 at 1st measurement had an increase of deduced stage, while 8 of 20 (40%) patients with deduced fibrosis stages of F2-4 at 1st measurement had a reduction of deduced stage. The factor associated with an increase of deduced fibrosis stage was lower baseline albumin levels ($P = 0.0092$).

The reason why the significant increase of LS was not detected in the natural course in these reports is

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 Score[42] 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 deposition[50] in the liver have been developed. Such as ActiTest,[47] SteatoTest,[49] and MR imaging for quantification of fat[48] and iron contents[50] in liver provide the information other than fibrosis derived from liver biopsy. Thus in the near future, non-invasive methods will replace liver biopsy.

REFERENCES

1 Forns X, Ampurdanes S, Llovet JM *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; **36**: 986–92.
2 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069–75.
3 Murawaki Y, Ikuta Y, Okamoto K, Koda M, Kawasaki H. Diagnostic value of serum markers of connective tissue turnover for predicting histological staging and grading in patients with chronic hepatitis C. *J Gastroenterol* 2001; **36**: 399–406.
4 Poynard T, Bedossa P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. METAVIR and CLINIVIR Cooperative Study Groups. *J Viral Hepat* 1997; **4**: 199–208.
5 Rosenberg WM, Voelker M, Thiel R *et al.* Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; **127**: 1704–13.
6 Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518–26.
7 Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. *Hepatology* 2007; **45**: 297–306.
8 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705–13.
9 Nitta Y, Kawabe N, Hashimoto S *et al.* Liver stiffness measured by transient elastography correlates with fibrosis area in liver biopsy in patients with chronic hepatitis C. *Hepatol Res* 2009; **39**: 675–84.
10 Friedrich-Rust M, Wunder K, Kriener S *et al.* Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. *Radiology* 2009; **252**: 595–604.
11 Friedrich-Rust M, Ong MF, Herrmann E *et al.* Real-time elastography for noninvasive assessment of liver fibrosis in chronic viral hepatitis. *AJR Am J Roentgenol* 2007; **188**: 758–64.
12 Yin M, Talwalkar JA, Glaser KJ *et al.* Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol* 2007; **5**: 1207–13. e2.
13 Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449–57.
14 Regev A, Berho M, Jeffers LJ *et al.* Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614–18.

15 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 8–13.
16 Kumada H, Okanoue T, Onji M *et al.* Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis B virus infection for the fiscal year 2008 in Japan. *Hepatol Res* 2010; **40**: 1–7.
17 Friedrich-Rust M, Ong MF, Martens S *et al.* Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960–74.
18 Yoshioka K, Kawabe N, Hashimoto S. Transient elastography: applications and limitations. *Hepatol Res* 2008; **38**: 1063–8.
19 Friedrich-Rust M, Schwarz A, Ong M *et al.* Real-time tissue elastography versus FibroScan for noninvasive assessment of liver fibrosis in chronic liver disease. *Ultraschall Med* 2009; **30**: 478–84.
20 Koizumi Y, Hirooka M, Kisaka Y *et al.* Liver fibrosis in patients with chronic hepatitis C: noninvasive diagnosis by means of real-time tissue elastography – establishment of the method for measurement. *Radiology* 2011; **258**: 610–17.
21 Takahashi H, Ono N, Eguchi Y *et al.* Evaluation of acoustic radiation force impulse elastography for fibrosis staging of chronic liver disease: a pilot study. *Liver Int* 2009; **30**: 538–45.
22 Fierbinteanu-Braticevici C, Andronescu D, Usvat R, Cretoiu D, Baicus C, Marinoschi G. Acoustic radiation force imaging sonoelastography for noninvasive staging of liver fibrosis. *World J Gastroenterol* 2009; **15**: 5525–32.
23 Lupsor M, Badea R, Stefanescu H *et al.* Performance of a new elastographic method (ARFI technology) compared to unidimensional transient elastography in the noninvasive assessment of chronic hepatitis C. Preliminary results. *J Gastrointestin Liver Dis* 2009; **18**: 303–10.
24 Sporea I, Sirli R, Popescu A, Danila M. Acoustic Radiation Force Impulse (ARFI) – a new modality for the evaluation of liver fibrosis. *Med Ultrasound* 2010; **12**: 26–31.
25 Toshima T, Shirabe K, Takeishi K *et al.* New method for assessing liver fibrosis based on acoustic radiation force impulse: a special reference to the difference between right and left liver. *J Gastroenterol* 2011; **46**: 705–11.
26 Ebinuma H, Saito H, Komuta M *et al.* Evaluation of liver fibrosis by transient elastography using acoustic radiation force impulse: comparison with Fibroscan((R)). *J Gastroenterol* 2011; **46**: 1238–48.
27 Sporea I, Sirli RL, Deleanu A *et al.* Acoustic radiation force impulse elastography as compared to transient elastography and liver biopsy in patients with chronic hepatopathies. *Ultraschall Med* 2011; **32** (Suppl 1): S46–52.
28 Grgurevic I, Cikara I, Horvat J *et al.* Noninvasive assessment of liver fibrosis with acoustic radiation force impulse imaging: increased liver and splenic stiffness in patients with liver fibrosis and cirrhosis. *Ultraschall Med* 2011; **32**: 160–6.
29 Piscaglia F, Salvatore V, Di Donato R *et al.* Accuracy of VirtualTouch Acoustic Radiation Force Impulse (ARFI) imaging for the diagnosis of cirrhosis during liver ultrasonography. *Ultraschall Med* 2011; **32**: 167–75.
30 Ichino N, Osakabe K, Nishikawa T *et al.* A new index for non-invasive assessment of liver fibrosis. *World J Gastroenterol* 2010; **16**: 4809–16.
31 Boursier J, Vergniol J, Sawadogo A *et al.* The combination of a blood test and Fibroscan improves the non-invasive diagnosis of liver fibrosis. *Liver Int* 2009; **29**: 1507–15.
32 Hayashi K, Katano Y, Honda T *et al.* Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy. *Liver Int* 2011 **31**: 1359–65.
33 Poynard T, Munteanu M, Colombo M *et al.* FibroTest is an independent predictor of virologic response in chronic hepatitis C patients retreated with pegylated interferon alfa-2b and ribavirin in the EPIC(3) program. *J Hepatol* 2011; **54**: 227–35.
34 Ogawa E, Furusyo N, Toyoda K, Takeoka H, Maeda S, Hayashi J. The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin. *Antiviral Res* 2009; **83**: 127–34.
35 Arima Y, Kawabe N, Hashimoto S *et al.* Reduction of liver stiffness by interferon treatment in the patients with chronic hepatitis C. *Hepatol Res* 2010; **40**: 383–92.
36 Wang JH, Changchien CS, Hung CH *et al.* Liver stiffness decrease after effective antiviral therapy in patients with chronic hepatitis C: longitudinal study using FibroScan. *J Gastroenterol Hepatol* 2010; **25**: 964–9.
37 Osakabe K, Ichino N, Nishikawa T *et al.* Reduction of liver stiffness by antiviral therapy in chronic hepatitis B. *J Gastroenterol* 2011; (in press).
38 Enomoto M, Mori M, Ogawa T *et al.* Usefulness of transient elastography for assessment of liver fibrosis in chronic hepatitis B: regression of liver stiffness during entecavir therapy. *Hepatol Res* 2010; **40**: 853–61.
39 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; **28**: 930–8.
40 Zaman A, Hapke R, Flora K, Rosen HR, Benner K. Factors predicting the presence of esophageal or gastric varices in patients with advanced liver disease. *Am J Gastroenterol* 1999; **94**: 3292–6.
41 Stefanescu H, Grigorescu M, Lupsor M *et al.* A new and simple algorithm for the noninvasive assessment of esophageal varices in cirrhotic patients using serum fibrosis markers and transient elastography. *J Gastrointestin Liver Dis* 2011; **20**: 57–64.

42 Lok AS, Ghany MG, Goodman ZD *et al.* Predicting cirrhosis in patients with hepatitis C based on standard laboratory tests: results of the HALT-C cohort. *Hepatology* 2005; 42: 282–92.
43 Jung KS, Kim SU, Ahn SH *et al.* Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). *Hepatology* 2011; 53: 885–94.
44 Masuzaki R, Tateishi R, Yoshida H *et al.* Risk assessment of hepatocellular carcinoma in chronic hepatitis C patients by transient elastography. *J Clin Gastroenterol* 2008; 42: 839–43.
45 Masuzaki R, Tateishi R, Yoshida H *et al.* Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; 49: 1954–61.
46 Harata M, Hashimoto S, Kawabe N *et al.* Liver stiffness in extrahepatic cholestasis correlates positively with bilirubin and negatively with alanine aminotransferase. *Hepatol Res* 2011; 41: 423–9.
47 Poynard T, Munteanu M, Ngo Y *et al.* ActiTest accuracy for the assessment of histological activity grades in patients with chronic hepatitis C, an overview using Obuchowski measure. *Gastroenterol Clin Biol* 2010; 34: 388–96.
48 Hatta T, Fujinaga Y, Kadoya M *et al.* Accurate and simple method for quantification of hepatic fat content using magnetic resonance imaging: a prospective study in biopsy-proven nonalcoholic fatty liver disease. *J Gastroenterol* 2010; 45: 1263–71.
49 Poynard T, Ratziu V, Naveau S *et al.* The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005; 4: 10.
50 Sirlin CB, Reeder SB. Magnetic resonance imaging quantification of liver iron. *Magn Reson Imaging Clin N Am* 2010; 18: 359–81. ix.

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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-α2b 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-α2b 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-α2b (1.5 μg/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 μl of serum using a commercial kit (QIAamp Viral RNA Kit; Qiagen, Valencia, CA) and dissolved in 50 μl 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-μl PCR reaction mixture contained 100 nM of each primer, 1 ng of template cDNA, 5 μl of GeneAmp 10× PCR buffer, 2 μl of dNTPs, and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA). Primers for the core region were: sense, 5′-GGGAGGTCTCGTAGACCGTGCACCATG-3′ and antisense, 5′-GAGMGGKATRTACCCCATGAGRTCGGC-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′-CTGCGTGAAGTGGTGGAATAC-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′-TACGCCGGGGGTCAKTRGGGCCCCA-3′; and for the NS5A-ISDR, sense 5′-TGTTTCCCCCACGCACTAC-3′ and antisense 5′-TGATGGGCAGTTTTTGTTCTTC-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)	*P*-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 ($10^4/\mu l$)	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 studied 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

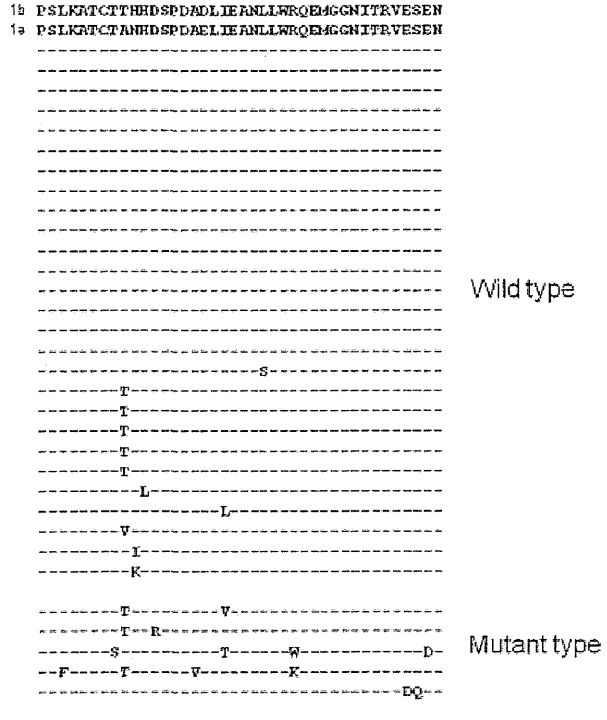

Fig. 1. Alignment of the amino acid sequence for the NS5A-ISDR. In the sequence alignment, dashes indicate amino acids identical to consensus sequence HCV1. Sequences of the HCV1 strain and HCV1 strains with one-nucleotide substitutions were defined as wild-type ISDR, and all other strains were defined 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 ($\times 10^4/mm^3$)	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-α2b and ribavirin in patients with HCV genotype 1a. Combined use of both IL28B and ISDR could improve the prediction of IFN response.

REFERENCES

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 46:1357–1364.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kumada H. 2009. Amino acid substitutions in the hepatitis C virus core region of genotype 1b are the important predictor of severe insulin resistance in patients without cirrhosis and diabetes mellitus. J Med Virol 81:1032–1039.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. 2010. Amino acid substitution in hepatitis C virus core region and genetic variation near the interleukin 28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 52:421–429.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. 2011. Amino acid substitution in HCV core/NS5A region and genetic variation near IL28B gene affect treatment efficacy to interferon plus ribavirin combination therapy. Intervirology (in press).

Alestig E, Arnholm B, Eilard A, Lagging M, Nilsson S, Norkrans G, Wahlberg T, Wejstål R, Westin J, Lindh M. 2011. Core mutations, IL28B polymorphisms and response to peginterferon/ribavirin treatment in Swedish patients with hepatitis C virus genotype 1 infection. BMC Infect Dis 12:124.

Dal Pero F, Tang KH, Gerotto M, Bortoletto G, Paulon E, Herrmann E, Zeuzem S, Alberti A, Naoumov NV. 2007. Impact of NS5A sequences of hepatitis C virus genotype 1a on early viral kinetics during treatment with peginterferon-alpha 2a plus ribavirin. J Infect Dis 196:998–1005.

Enomoto N, Takada A, Nakao T, Date T. 1990. There are two major types of hepatitis C virus in Japan. Biochem Biophys Res Commun 170:1021–1025.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Fujimura Y, Ishimoto S, Shimoyama T, Narita N, Kuze Y, Yoshioka A, Fukui H, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. 1996. Genotypes and multiple infections with hepatitis C virus in patients with haemophilia A in Japan. J Viral Hepat 3:79–84.

Gale M, Jr., Blakely CM, Kwieciszewski B, Tan SL, Dossett M, Tang NM, Korth MJ, Polyak SJ, Gretch DR, Katze MG. 1998. Control of PKR protein kinase by hepatitis C virus nonstructural 5A protein: Molecular mechanisms of kinase regulation. Mol Cell Biol 18:5208–5218.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Ghany MG, Strader DB, Thomas DL, Seeff LB, American Association for the Study of Liver Diseases. 2009. Diagnosis, management, and treatment of hepatitis C: An update. Hepatology 49: 1335–1374.

Hayashi K, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayakawa T, Morita K, Nishimura D, Kato K, Urano F, Takamatsu J. 2003. Prevalence and characterization of hepatitis C virus genotype 4 in Japanese hepatitis C carriers. Hepatol Res 25:409–414.

Hayashi K, Katano Y, Takeda Y, Honda T, Ishigami M, Itoh A, Hirooka Y, Nakano I, Yano M, Goto H, Yoshioka K, Toyoda H, Kumada T. 2007. Comparison of hepatitis B virus subgenotypes in patients with acute and chronic hepatitis B and absence of lamivudine-resistant strains in acute hepatitis B in Japan. J Med Virol 79:366–373.

Hayashi K, Katano Y, Ishigami M, Itoh A, Hirooka Y, Nakano I, Urano F, Yoshioka K, Toyoda H, Kumada T, Goto H. 2011a. Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy. J Viral Hepat 18:280–286.

Hayashi K, Katano Y, Honda T, Ishigami M, Itoh A, Hirooka Y, Ishikawa T, Nakano I, Yoshioka K, Toyoda H, Kumada T, Goto H. 2011b. Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy. Liver Int 9:1359–1365.

Hayes CN, Kobayashi M, Akuta N, Suzuki F, Kumada H, Abe H, Miki D, Imamura M, Ochi H, Kamatani N, Nakamura Y, Chayama K. 2011. HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy. Gut 60:261–267.

Herion D, Hoofnagle JH. 1997. The interferon sensitivity determining region: All hepatitis C virus isolates are not the same. Hepatolgy 25:769–770.

Hofgärtner WT, Polyak SJ, Sullivan DG, Carithers RL, Jr., Gretch DR. 1997. Mutations in the NS5A gene of hepatitis C virus in North American patients infected with HCV genotype 1a or 1b. J Med Virol 53:118–126.

Kumada H, Okanoue T, Onji M, Moriwaki H, Izumi N, Tanaka E, Chayama K, Sakisaka S, Takehara T, Oketani M, Suzuki F, Toyota J, Nomura H, Yoshioka K, Seike M, Yotsuyanagi H, Ueno Y, The Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis, Ministry of Health, Labour and Welfare of Japan. 2010. Guidelines for the treatment of chronic hepatitis and cirrhosis due to hepatitis C virus infection for the fiscal year 2008 in Japan. Hepatol Res 40: 8–13.

Kumthip K, Pantip C, Chusri P, Thongsawat S, O'Brien A, Nelson KE, Maneekarn N. 2011. Correlation between mutations in the core and NS5A genes of hepatitis C virus genotypes 1a, 1b, 3a, 3b, 6f and the response to pegylated interferon and ribavirin combination therapy. J Viral Hepat 18:e117–e125.

Kurosaki M, Tanaka Y, Nishida N, Sakamoto N, Enomoto N, Honda M, Sugiyama M, Matsuura K, Sugauchi F, Asahina Y, Nakagawa M, Watanabe M, Sakamoto M, Maekawa S, Sakai A, Kaneko S, Ito K, Masaki N, Tokunaga K, Izumi N, Mizokami M. 2011. Pretreatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in IL28B and viral factors. J Hepatol 54:439–448.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, Kuramitsu T, Kawata S, Tanaka E, Izumi N, Okuse C, Kakumu S, Okanoue T, Hino K, Hiasa Y, Sata M, Maeshiro T, Sugauchi F, Nojiri S, Joh T, Miyakawa Y, Mizokami M. 2009. Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. J Clin Microbiol 47:1476–1483.

McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS, IDEAL Study Team. 2009. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 361:580–593.

Nakagawa M, Sakamoto N, Ueyama M, Mogushi K, Nagaie S, Itsui Y, Azuma S, Kakinuma S, Tanaka H, Enomoto N, Watanabe M. 2010. Mutations in the interferon sensitivity determining region and virological response to combination therapy with pegylated-interferon alpha 2b plus ribavirin in patients with chronic hepatitis C-1b infection. J Gastroenterol 45:656–665.

Nakano I, Fukuda Y, Katano Y, Nakano S, Kumada T, Hayakawa T. 1999. Why is the interferon sensitivity-determining region (ISDR) system useful in Japan? J Hepatol 30:1014–1022.

Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H. 2009. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for

hepatitis C: A Japanese multi-center study. J Gastroenterol 44: 952–963.

Otagiri H, Fukuda Y, Nakano I, Katano Y, Toyoda H, Yokozaki S, Hayashi K, Hayakawa T, Fukuda Y, Kinoshita M, Takamatsu J. 2002. Evaluation of a new assay for hepatitis C virus genotyping and viral load determination in patients with chronic hepatitis C. J Virol Methods 103:137–143.

Pascu M, Martus P, Höhne M, Wiedenmann B, Hopf U, Schreier E, Berg T. 2004. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: A meta-analysis focused on geographical differences. Gut 53:1345–1351.

Sakugawa H, Nakasone H, Kinjo F, Saito A, Keida Y, Kikuchi K, Oyadomari Y, Ishihara M, Nakasone K, Yogi S, Kinjo Y, Taira M. 1997. Clinical features of patients with chronic liver disease associated with hepatitis C virus genotype 1a/I in Okinawa, Japan. J Gastroenterol Hepatol 12:176–181.

Seeff LB. 2002. Natural history of chronic hepatitis C. Hepatology 36:S35–S46.

Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S, Halfon P, Inchauspé G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. 2005. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 42:962–973.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1104.

Tachi Y, Katano Y, Honda T, Hayashi K, Ishigami M, Itoh A, Hirooka Y, Nakano I, Samejima Y, Goto H. 2010. Impact of amino acid substitutions in the hepatitis C virus genotype 1b core region on liver steatosis and hepatic oxidative stress in patients with chronic hepatitis C. Liver Int 30:554–559.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. 2009. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 461:798–801.

Toyoda H, Kumada T, Tada T, Arakawa T, Hayashi K, Honda T, Katano Y, Goto H. 2010. Association between HCV amino acid substitutions and outcome of peginterferon and ribavirin combination therapy in HCV genotype 1b and high viral load. J Gastroenterol Hepatol 25:1072–1078.

Yahoo N, Sabahi F, Shahzamani K, Malboobi MA, Jabbari H, Sharifi H, Mousavi-Fard SH, Merat S. 2011. Mutations in the E2 and NS5A regions in patients infected with hepatitis C virus genotype 1a and their correlation with response to treatment. J Med Virol 83:1332–1337.

Yokozaki S, Katano Y, Hayashi K, Ishigami M, Itoh A, Hirooka Y, Nakano I, Goto H. 2011. Mutations in two PKR-binding domains in chronic hepatitis C of genotype 3a and correlation with viral loads and interferon responsiveness. J Med Virol 83:1727–1732.

Zeuzem S, Lee JH, Roth WK. 1997. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alfa. Hepatology 25:740–744.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

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/μL) 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/μL 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/μL) 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/μL). 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 Cockroft–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/μL at week 2 (Fig. 1). Eltrombopag treatment was to be discontinued if platelet counts were >200,000/μL 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 ≥80,000/μL) 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

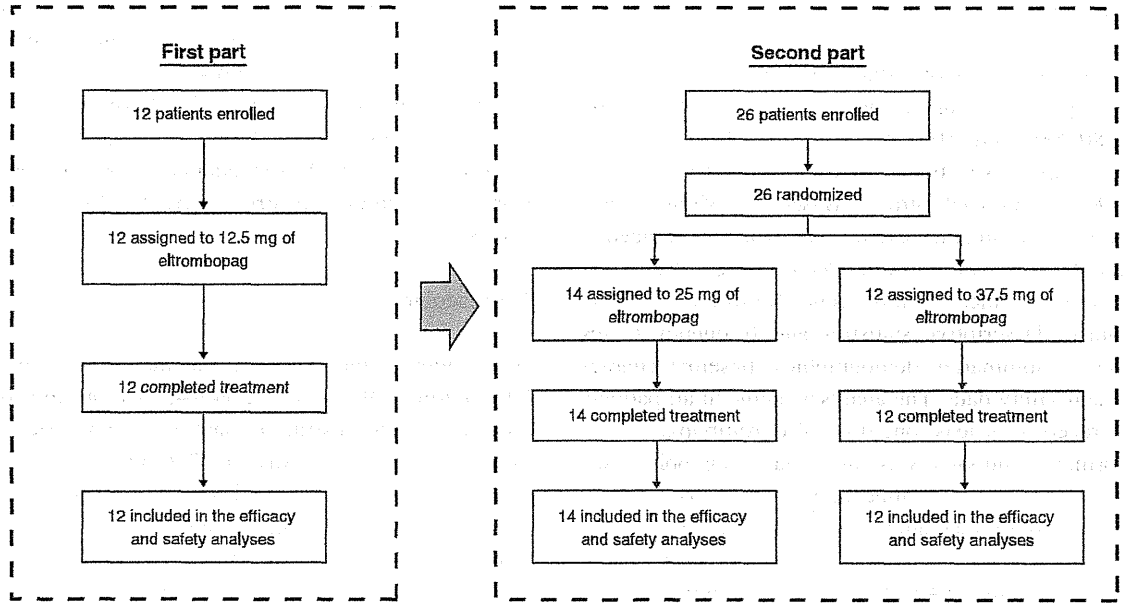

Fig. 1 Study design. The study was a multicenter, open-label, dose-ranging phase II study that used a unique sequential design and consisted of 2 parts. After review, by a Safety Review Committee, of safety data from the 12.5 mg group (first part), new patients were randomly assigned to receive 25 or 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 $AUC_{0-\tau}$] 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/μL in the 12.5, 25, and 37.5 mg groups, respectively, and the standard deviation was 50,000/μL 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 ≥80,000/μL) 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/μL 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/μL for 1 week thereafter (Fig. 4). The median increases from baseline in platelet counts at week 2 for

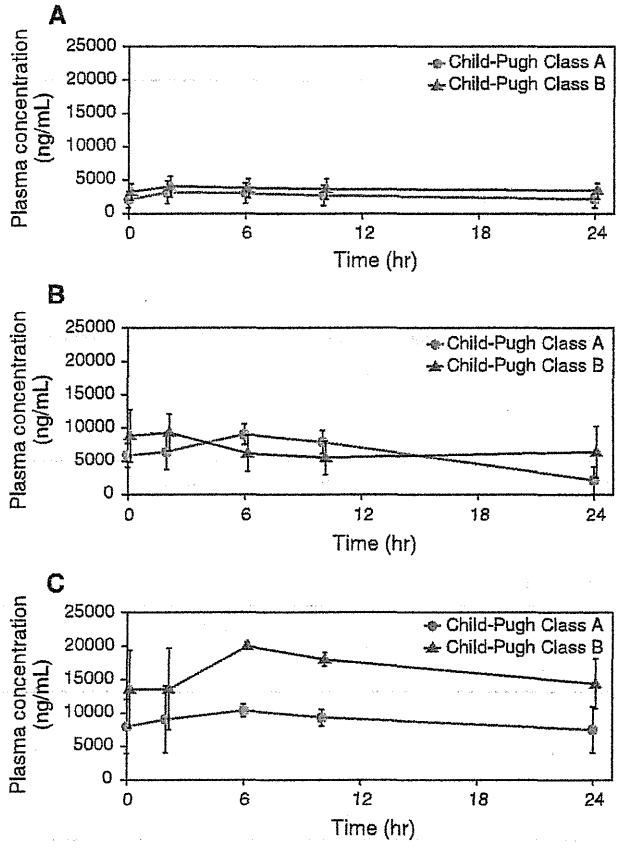

Fig. 2 Plasma eltrombopag concentration stratified by Child–Pugh class in the 12.5 mg (**a**), 25 mg (**b**), and 37.5 mg groups (**c**). One patient in the 12.5 mg group with Child–Pugh class A and another patient in the 37.5 mg group with Child–Pugh class B were excluded from summary statistics of 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.

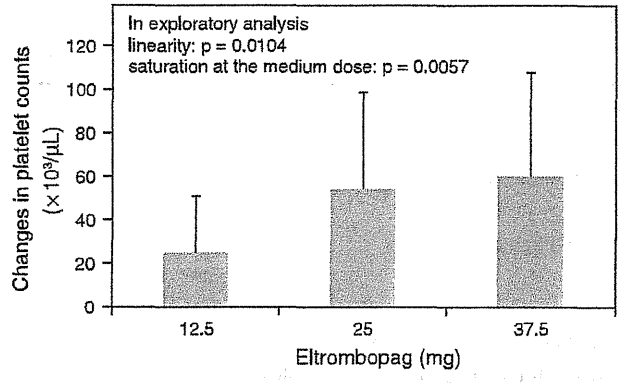

Fig. 3 Changes from baseline in platelet counts at week 2. Exploratory analyses were conducted to detect a dose response and trend, using the changes from baseline in platelet counts at week 2. These data were analyzed using analysis of covariance (ANCOVA) with baseline platelet counts as a covariate, 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

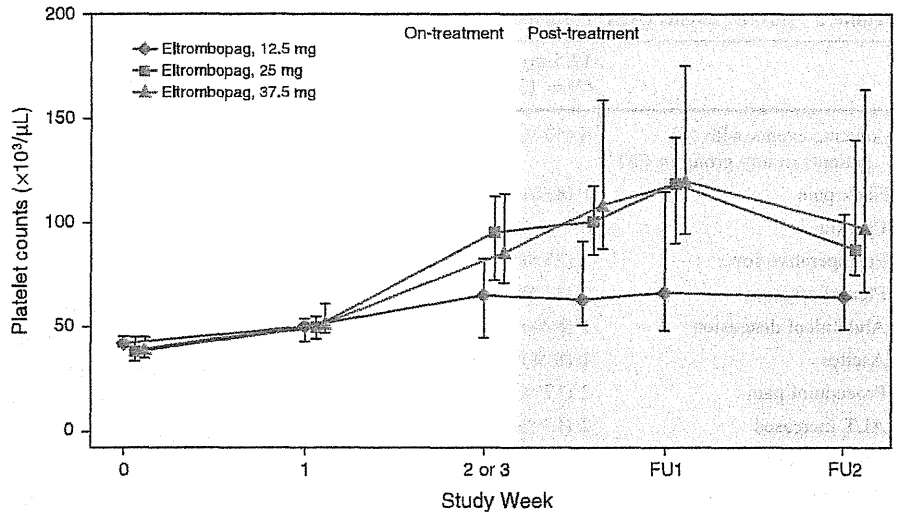

Fig. 4 Median platelet counts after treatment with eltrombopag. Platelet counts at either week 2 or 3, or at the end of treatment with eltrombopag. Platelet counts after the end of treatment include the values after invasive procedures or platelet transfusions. Data are expressed as medians with interquartile 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/μL 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 $AUC_{0-\tau}$ in Japanese patients receiving 37.5 mg of eltrombopag once daily was estimated to be 236 μg 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