Original Research

Presence of a Hypovascular Hepatic Nodule Showing Hypointensity on Hepatocyte-Phase Image Is a Risk Factor for Hypervascular Hepatocellular Carcinoma

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Purpose: To determine whether the presence of a hypovascular nodule in the liver showing hypointensity on hepatocyte-phase of gadoxetic acid-enhanced magnetic resonance imaging (EOB-MRI) is a risk factor for hypervascular hepatocellular carcinoma (HCC) in patients with chronic liver disease.

Materials and Methods: Forty-one patients with pathologically confirmed hypervascular HCC and 41 age- and gender-matched controls were retrospectively selected. These patients had undergone EOB-MRI at least twice: the latest EOB-MRI and EOB-MRI performed more than 6 months earlier. History of hypervascular HCC, presence of a hypointense hypovascular nodule in previous hepatocyte-phase MR images, percent prothrombin time, platelet count, serum levels of albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, α-fetoprotein, and protein induced by vitamin K absence-II (PIVKA-II) were variables evaluated by multivariate logistic regression analysis.

Results: Multivariate analysis revealed that serum albumin level (odds ratio [95% confidence interval], 0.19 [0.06–0.57]; $P = 0.0024$), history of hypervascular HCC (8.62 [2.71–32.8]; $P = 0.0001$), and presence of a hypointense hypovascular nodule (4.18 [1.18–17.2]; $P = 0.0256$) were significant risk factors for hypervascular HCC.

Conclusion: Patients with chronic liver disease showing a hypointense hypovascular nodule in the liver on hepatocyte-phase EOB-MRI have a high risk of HCC development.

Key Words: magnetic resonance imaging; gadoxetic acid; hepatocyte phase; hepatocellular carcinoma; risk factors
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IN THE CIRRHOTIC LIVER, hepatocellular carcinoma (HCC) develops by multistep hepatocarcinogenesis from a dysplastic nodule, through early HCC, to advanced HCC (1). The 5-year survival rate is higher among patients with early HCC than among those with advanced HCC. Therefore, risk assessment of HCC is essential for managing patients with chronic liver disease.

Early HCC may be detected as a hypointense hypovascular nodule in the cirrhotic liver by hepatocyte-phase gadoxetic acid-enhanced magnetic resonance imaging (EOB-MRI) (2). Recent studies have revealed a high incidence of hypervascularization in nonhypervascular nodules showing hypointensity in earlier hepatocyte-phase EOB-MR images (3–5). Such hypervascularization indicates the possibility of hypervascular HCC development. However, patients with these nodules frequently develop HCC in parts of the liver where no nodule was previously observed by EOB-MRI. Therefore, we hypothesized that patients with hypointense hypovascular nodules in the liver observed by hepatocyte-phase EOB-MRI might have a high risk of HCC development and could develop hypervascular HCC not only from the hypovascular nodules but also from any part of the liver.

To validate this hypothesis, we investigated whether the presence of a hypovascular nodule showing hypointensity on hepatocyte-phase EOB-MRI is a risk factor for hypervascular HCC in patients with chronic liver disease.

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Table 1
Patient Demographic Data

Parameter	With HCC	Without HCC	P
Number of patients	41	41	
Mean age (SD)	70.1 (7.29)	70.0 (7.28)	
Men:women	26:15	26:15	
Interval [days] (SD)	337 (202)	333 (143)	0.4896
Liver disease			0.4313
Viral hepatitis type C	29	28	
Viral hepatitis type B	5	5	
Alcoholic	4	1	
Other	3	7	

Interval means the duration between the previous MRI and current MRI.
Interval was compared by Mann-Whitney *U*-test and liver disease was compared by the χ^2 test.

MATERIALS AND METHODS

Subjects

This retrospective case–control study was performed in accordance with the principles of the Declaration of Helsinki. The institutional Ethics Committee approved the study protocol and waived the need for written informed consent from the subjects.

First, a radiologist with 8 years of experience reviewed the records of MR examinations performed at our institute from January 2008 to April 2012 to identify patients with chronic liver disease who had undergone EOB-MRI. If more than one EOB-MRI was performed per patient, the latest examination was considered current MRI. EOB-MRI for staging or screening of liver cancer over 6 months earlier was considered previous MRI. From the records of patients who had undergone both previous and current MRI ($n = 746$), the radiologist selected the current MRI reports that suggested hypervascular HCC ($n = 220$). Patients with suspected HCC on EOB-MRI but without pathological confirmation ($n = 151$) and those with other hepatic masses (metastasis, $n = 13$; cholangiocarcinoma, $n = 15$) were excluded. Finally, 41 patients (26 men and 15 women; age range, 54–85 years, mean age, 70.1 years) were included as cases (with-HCC group) in this study. HCC had been pathologically confirmed by partial hepatectomy ($n = 24$) or percutaneous needle biopsy ($n = 17$). No tumor was found in the liver of the remaining 526 patients by current MRI; 41 age- and gender-matched controls (without-HCC group) were selected from these patients (Table 1).

EOB-MRI

EOB-MRI had been performed in all patients using a superconducting magnet operating at 1.5 T (Signa EXCITE HD; GE Medical Systems, Milwaukee, WI) and an 8-channel phased-array coil. Dynamic fat-suppressed gradient-echo T1-weighted images with a 3D acquisition sequence (liver acquisition with volume acceleration) had been obtained before (precontrast) and at 20–30 seconds (arterial phase, scan timing was adjusted by using the fluoroscopic triggering technique), and 1 (portal venous phase), 2 (late phase), 5, 10, and 20 minutes (hepatocyte phase) after the administration of gadoxetic acid (EOB Primovist; Bayer HealthCare, Osaka, Japan). The contrast material (0.025 mmol/kg body weight) had been administered as an intravenous bolus at a rate of 1 mL/s via an intravenous cubital line (with 20 or 22G), which had been flushed with 20 mL saline using a power injector (Sonic Shot 50; Nemoto Kyorindo, Tokyo, Japan). Hepatocyte-phase images of 20 minutes acquired in the transverse and sagittal planes were used for evaluation in this study. A section thickness of 5 mm and a 2.5 mm overlap (ie, 2.5 mm interval) were applied. The repetition time / echo time ratio was 3.8/1.9 msec; flip angle, 12°; number of signals acquired, 1; field of view, 35–42 × 40–45 cm; matrix size, 320 × 192; acquisition time, 18 seconds; and parallel imaging (ASSET) factor, 1.75.

Statistical Analyses

The following variables were analyzed as potential risk factors of HCC development: serum albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase (ALT), α-fetoprotein, and protein induced by vitamin K absence-II levels; percent prothrombin time and platelet count; history of hypervascular

Table 2
Results of the Univariate Analysis

Parameter	With HCC	Without HCC	P
Albumin (g/dL)	3.7 (3.7, 2.7–4.8)	4.0 (4.0, 2.9–4.9)	0.0176
Total bilirubin (mg/dL)	0.8 (0.7, 0.3–3.2)	0.7 (0.6, 0.2–2.1)	0.4895
Aspartate aminotransferase (U/L)	50 (44, 21–146)	42 (39, 16–98)	0.1389
Alanine aminotransferase (U/L)	41 (35, 10–162)	38 (29, 9–117)	0.3857
Percent prothrombin time (%)	75.1 (71.7, 51.4–104)	80.4 (78.9, 53.5–119.9)	0.0591
Platelet count (10^9/L)	117 (97, 48–377)	137 (128, 52–356)	0.1957
α-Fetoprotein (ng/mL)	46.3 (10.4, 1.8–640)	17.3 (4.3, 0.9–256)	0.0001
Protein induced by vitamin K absence-II (mAU/mL)	397 (17, 4–15135)	29 (17, 9–338)	0.5578
History of hypervascular HCC	31/41 (75.6%)	16/41 (39.0%)	0.0008
Presence of hypointense hypovascular nodule in hepatocyte-phase EOB-MR images	15/41 (36.6%)	5/41 (12.2%)	0.0101

Serum marker levels were analyzed by the Mann–Whitney *U*-test and are expressed as means (median, range). Categorical variables were analyzed by the χ^2 test and are represented as n/N (%).

Table 3
Results of the Multivariate Analysis

Parameter	*P*	Odds ratio (95% confidence interval)
Age (per year)[a]	0.2544	0.96 (0.88–1.03)
Albumin (per 1.0 g/dL)	0.0024	0.19 (0.06–0.57)
α-Fetoprotein (per 1.0 ng/mL)	0.6481	1.00 (0.99–1.02)
History of hypervascular HCC	0.0001	8.62 (2.71–32.8)
Presence of hypointense hypovascular nodule in hepatocyte-phase EOB-MR images	0.0256	4.18 (1.18–17.2)

Although age-matched controls were selected, patient age was added as a variable to ensure the exclusion of an age effect.

HCC; and presence of a hypointense hypovascular nodule in hepatocyte-phase images obtained by previous MRI, which included nodules that were considered hypervascular HCC on current MRI and those that remained hypovascular on current MRI with or without increasing in size.

Categorical and continuous variables were compared using the χ^2 test and Mann–Whitney *U*-test, respectively. For multivariate analysis, the odds ratio (OR) was estimated by logistic regression analysis using age and the variables showing significant differences between the with-HCC and the without-HCC groups in the univariate analysis. Although age-matched controls were selected, patient age was added as a variable to ensure the exclusion of an age effect for enhancing the adjustment of age between the groups. Data analysis was performed by using JMP software v. 10 (SAS Institute Japan, Tokyo, Japan). A two-sided *P*-value of less than 0.05 was considered significant.

RESULTS

Univariate Analysis

The with-HCC and without-HCC groups showed significant differences in presence of a hypointense hypovascular nodule in hepatocyte-phase images obtained by previous MRI (*n*/*N* [%], 15/41 [36.6%] vs. 5/41 [12.2%]; $P = 0.0101$), serum albumin level (median [range], 3.7 [2.7–4.8] vs. 4.0 [2.9–4.9] g/dL; $P = 0.0176$), serum α-fetoprotein level (10.4 [1.8–640] vs. 4.3 [0.9–256] ng/mL; $P = 0.0001$), and history of hypervascular HCC (31/41 [75.6%] vs. 16/41 [39.0%]; $P = 0.0008$) (Table 2).

Multivariate Logistic Regression Analysis

Serum albumin level (OR [95% confidence interval], 0.19 [0.06–0.57]; $P = 0.0024$), history of hypervascular HCC (8.62 [2.71–32.8]; $P = 0.0001$), and presence of a hypointense hypovascular nodule in hepatocyte-phase images obtained by previous MRI (4.18 [1.18–17.2]; $P = 0.0256$) were significant risk factors for hypervascular HCC (Table 3, Fig. 1).

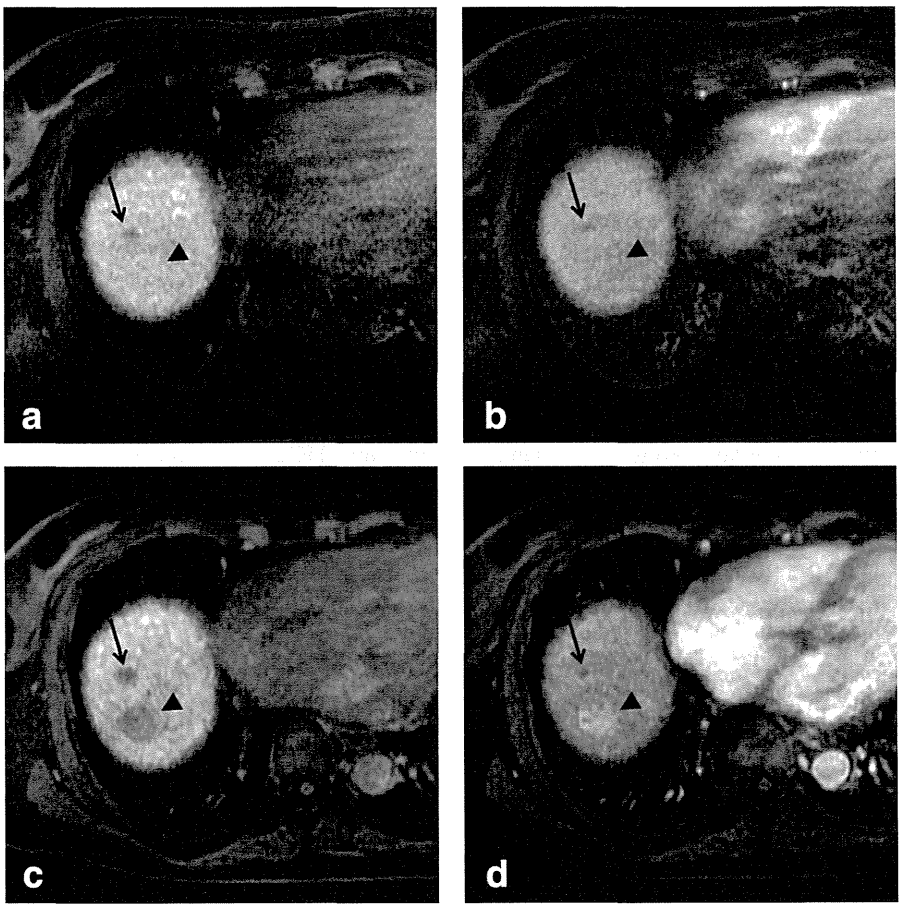

Figure 1. Findings in a 70-year-old woman with viral hepatitis type C. There was a hypointense nodule in S8 measuring 10 mm on hepatocyte-phase EOB-MR image of previous MRI (arrow). This nodule did not show hypervascularity on the arterial-phase image of previous MRI (arrow). This nodule slightly increased in size, measuring 12 mm (arrow), and another hypointense lesion measuring 20 mm is visible behind the nodule (arrowhead) of current MRI. The first nodule does not show hypervascularity on the arterial-phase image (arrow), whereas the second nodule shows hypervascularity (arrowhead) on current MRI. The second nodule could not be detected by previous MRI (arrowhead). **a:** Hepatocyte-phase EOB-MR image of previous MRI. **b:** Arterial-phase image of previous MRI. **c:** Hepatocyte-phase EOB-MR image of current MRI. **d:** Arterial-phase image of current MRI.

DISCUSSION

In this study we investigated whether the presence of a hypovascular nodule showing hypointensity on hepatocyte-phase EOB-MRI is a risk factor for hypervascular HCC in patients with chronic liver disease. The results of the multivariate analysis validate our hypothesis that patients with hypointense hypovascular nodules in the liver observed by EOB-MRI might have a high risk of HCC development. We also found that the serum albumin level and a history of hypervascular HCC are independent risk factors for hypervascular HCC. 63.4% (26/41) of the hypervascular HCCs were not found on previous MRI. If we took MRI by thinner slice (ie, 1 mm), more lesion may be detected on previous MRI. Some of cases had a long interval (ie, 1143 days, 954 days, or 678 days). Some nodules might be visible provided a shorter-interval MRI.

Recent advances in imaging techniques may enable earlier and more accurate diagnosis of HCC (6–9). Gadoxetic acid, a liver-specific contrast agent that is retained by hepatocytes and excreted into the bile ducts (10,11), allows monitoring of small lesions over a long period. Hypovascular nodules in the cirrhotic liver that are found incidentally in routine clinical MRI scans tend to show low signal intensity, indicative of poor gadoxetic acid uptake, on hepatocyte-phase EOB-MRI (3,11–13). Such nodules are now considered early HCC or high-risk lesions for developing hypervascular HCC (14). If such a lesion occurs, it will be thought that other parts of the liver are also likely to potentially develop HCC. Such a concept is well known in oral cancer as "field cancerization" (15).

The reported risk factors for HCC include older age, male gender, heavy alcohol intake, cirrhosis, low platelet count, high serum α-fetoprotein level, low serum albumin level, high serum ALT level, and poor response to locoregional treatments (16–23). Of these, patient age and gender are unanimously considered the strongest risk factors for HCC. Because the effect of the presence of a hypointense hypovascular nodule in hepatocyte-phase EOB-MR images on HCC development might be obscured by other known risk factors, including age and gender, we attempted to account for these confounding factors by using an age- and gender-matched control group.

Not only hepatologists but also radiologists should have knowledge about the risk factors for HCC, because they are expected to evaluate images that serve as a basis for patient management (eg, to recommend the frequency of imaging). Liver screening by efficient imaging would help to identify HCC in its early stages and improve the prognosis of patients with chronic liver disease (24).

Our study was mainly limited by its retrospective design and small number of patients. A prospective study with uniform subjects would be necessary to confirm the usefulness of the presence of a hypovascular hepatic nodule showing hypointensity on hepatocyte-phase EOB-MRI as a risk factor for HCC.

In conclusion, our retrospective case–control study revealed that patients with chronic liver disease showing a hypointense hypovascular nodule in the liver on hepatocyte-phase EOB-MRI have a high risk of hypervascular HCC development. EOB-MRI is a potential tool to select such patients for subsequent MR or computed tomographic examination within a short interval.

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Hepatology Research

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Original Article

Impaired brain activity in cirrhotic patients with minimal hepatic encephalopathy: Evaluation by near-infrared spectroscopy

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Aim: Near-infrared spectroscopy (NIRS) is a tool that could non-invasively measure the regional cerebral oxygenated hemoglobin (oxy-Hb) concentration with high time resolution. The aim of the present study is to reveal the time-dependent regional cerebral oxy-Hb concentration change coupled with brain activity during task performance in patients with minimal hepatic encephalopathy (MHE).

Methods: Cerebral oxy-Hb concentration was measured by using NIRS in 29 cirrhotic patients without overt hepatic encephalopathy (HE). Of those, 16 patients who had abnormal electroencephalography findings were defined as having MHE. Responsive increase in oxy-Hb during a word-fluency task was compared between MHE and non-MHE patients.

Results: There was no difference in the maximum value of oxy-Hb increase between patients with and without MHE (0.26 ± 0.12 vs 0.32 ± 0.22 mM·mm, $P = 0.37$). However, the pattern of the time course changes of oxy-Hb was different between the two groups. The MHE group was characterized by a gradual increase of oxy-Hb throughout the task compared to steep and repetitive increase in the non-MHE group. Increase in oxy-Hb concentration at 5 s after starting the task was significantly small in the MHE group compared to the non-MHE (0.03 ± 0.05 vs 0.11 ± 0.09 mM·mm, $P = 0.006$).

Conclusion: The cerebral oxygen concentration is poorly reactive in response to tasks among cirrhotic patients without overt HE but having abnormal electroencephalography findings. These impaired responses in regional cerebral oxy-Hb concentration may be related to the latent impairment of brain activity seen in MHE.

Key words: hepatic encephalopathy, near-infrared spectroscopy

INTRODUCTION

HEPATIC ENCEPHALOPATHY (HE) is a major complication of liver cirrhosis. Apart from clinically overt HE (OHE), minimal HE (MHE) is troublesome because it is associated with reduced quality of life (QOL), reduced cognitive function, lowered work efficiency, higher risk of progression to OHE and may be a cause of traffic accidents.[1–3] MHE treatment can improve QOL, driving capability and progression of OHE.[4–6] Adequate diagnosis of MHE and early therapeutic intervention are precluded by the lack of reliable diagnostic standards, and HE is usually diagnosed only after the presentation of overt symptoms. For the diagnosis of MHE, neuropsychological function tests, such as number connection test, light/sound reaction time, inhibitory control test, Wechsler adult intelligence scale (WAIS) or electro-psychological tests

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Conflict of interest: The authors who participated in this study have had no affiliation with the manufacturers of the drugs involved either in the past or at present, and have not received funding from the manufacturers to conduct this research.

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including electroencephalography (EEG), cerebral evoked potential, p300 event-related potential, psychometric hepatic encephalopathy score (PHES) and critical flicker test[7–15] have been employed. Diagnostic specificity can be improved by combining these tests, but complexity becomes a major disadvantage.

Recent advances in diagnostic imaging, such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), made it possible to map brain function in tomographic images with high space and time resolutions. Recent study using PET[16] revealed that the primary event in the pathogenesis of OHE is inhibition of cerebral energy metabolism evidenced by reduced cerebral oxygen consumption and reduced cerebral blood flow. Whether the same mechanism could be applied to MHE is not known. Near-infrared spectroscopy (NIRS) is a tool that could non-invasively measure the cerebral blood volume as an oxygenated hemoglobin (oxy-Hb) concentration. The space and time resolution of NIRS is equivalent or higher than that of PET and fMRI. Moreover, NIRS is highly portable, does not have any restriction in the posture and flexible in setting tasks. Therefore it is possible to perform tests in a natural environment and to evaluate brain function as reflected by the dynamic changes in regional cerebral oxy-Hb concentration in response to a given task. The latter may be especially important to disclose a latent abnormality of brain function.

Recent study suggested that astrocytes regulate the cerebral blood flow and provide the oxy-Hb to the activation site of the brain.[17–19] In hepatic encephalopathy patients, function of astrocyte is impaired which may lead to cerebral oxygen consumption and blood flow.[16,20–22] We hypothesized that clinically latent abnormality of brain function in MHE also may be linked to the impairment of adequate increase in cerebral energy metabolism in response to the stimulation for activating the brain due to impaired function of astrocytes. In the present study, we used NIRS to evaluate the latent abnormality of brain function in patients with MHE, by measuring the increase of regional cerebral oxy-Hb concentration in response to task stimulation.

METHODS

Patients

A TOTAL OF 29 liver cirrhosis patients without OHE were enrolled. The underlying etiology of liver disease was hepatitis C virus infection in 19 patients, hepatitis B virus infection in two, alcoholic liver disease in five and other liver disease in three. All participants were examined by two psychiatrists to exclude mental disorders. No patient had any history of taking antidepressants or other psychotropic drugs. Subjects were examined by brain MRI or brain CT and they had no apparent brain structural disease including brain infarction. The study was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of Musashino Red Cross Hospital and National Center of Neurology and Psychiatry. Informed consent was obtained from each subject. MHE was defined as those who had abnormal EEG findings. According to this definition, 16 patients were assigned to the MHE group and 13 were assigned to the non-MHE group. Table 1 shows the clinical characteristics of patients. The age and sex ratio did not differ between groups.

NIRS measurements

Concentration of oxy-Hb was measured by a 52-channel NIRS machine (Hitachi ETG4000; Hitachi Medical,

Table 1 Patient characteristics

	MHE ($n = 16$)	Non-MHE ($n = 13$)	*P*-value
Age	67.9 ± 8.9	70.1 ± 10.2	0.53
Sex (M/F)	7/9	7/6	0.72
Albumin (g/dL)	2.68 ± 0.39	3.63 ± 0.47	<0.0001
T-Bil (mg/dL)	1.83 ± 1.22	0.88 ± 0.34	0.011
PT%	64.5 ± 10.8	85.2 ± 12.7	<0.0001
Child–Pugh (A/B/C)	0/9/7	11/2/0	<0.0001
Etiology (HC/HB/Alc/Others)	8/2/4/2	11/0/1/1	0.28
NH3 (mmol/L)	90.1 ± 64.3	40.1 ± 18.3	0.012

Alc, alcoholic liver disease; HB, hepatitis B; HC, hepatitis C; MHE, minimal hepatic encephalopathy; PT%, prothrombin time percentage; T-Bil, total bilirubin.

Tokyo, Japan). NIRS detects changes in brain activity by capturing increases in regional cerebral blood flow caused by neural activity. For each channel, an optic fiber device is connected to an application probe that is placed on the subject's scalp. The 52 channels cover the frontal lobe, upper temporal lobe and anterior parietal lobe of the brain (Fig. 1). The near-infrared light penetrates the scalp and skull, passes through the brain tissue, and is partially absorbed by oxy-Hb. The reflected light is detected by a probe positioned 30 mm away from the application probe. The changes in concentration of oxy-Hb can be calculated by measuring reflected light.[23] In this study, the results measured by the seven channels which were previously reported to be diagnostic for mental disorders; (channels 36–38 and 46–49)[24–26] were selected for the analysis. The time-dependent changes in oxy-Hb concentration in each of these seven channels were compared between MHE and non-MHE patients. The sum of increase in oxy-Hb concentration in these seven channels was calculated and compared between MHE and non-MHE patients. For this analysis, increase of oxy-Hb at 5 s and maximum increase were used.

Activation task

A word-fluency task was used to stimulate frontal lobe activity. Subjects were instructed to generate as many words as possible with a given letter. For example, with a task involving "naming words starting with the letter 'T' ", subjects were given 20 s to say as many words as they could starting with the letter "T", such as "tomato", "tail" and "tea". Three tasks were presented for a total of 60 s. During the word-fluency test, the real-time changes in the oxy-Hb concentration were measured at each channel. Data are expressed as a wave form as well as in the form of topographic images.

Statistical analysis

The SPSS software package ver. 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Categorical data were analyzed using Fisher's exact test. Continuous variables were compared with Student's *t*-test. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

THE NUMBER OF words generated by the word-fluency task did not differ significantly between the MHE and non-MHE groups (10.8 ± 3.4 vs 10.7 ± 2.5 words, $P = 0.93$). Figure 2 shows the time-dependent changes in the oxy-Hb concentration during the task in the representative seven channels. The average value of the seven channels (36–38 and 46–49) is shown in Figure 2. These changes reflected frontal lobe activation by the word-fluency test and correspondingly elevated cerebral blood flow in the frontal lobe. In the non-MHE

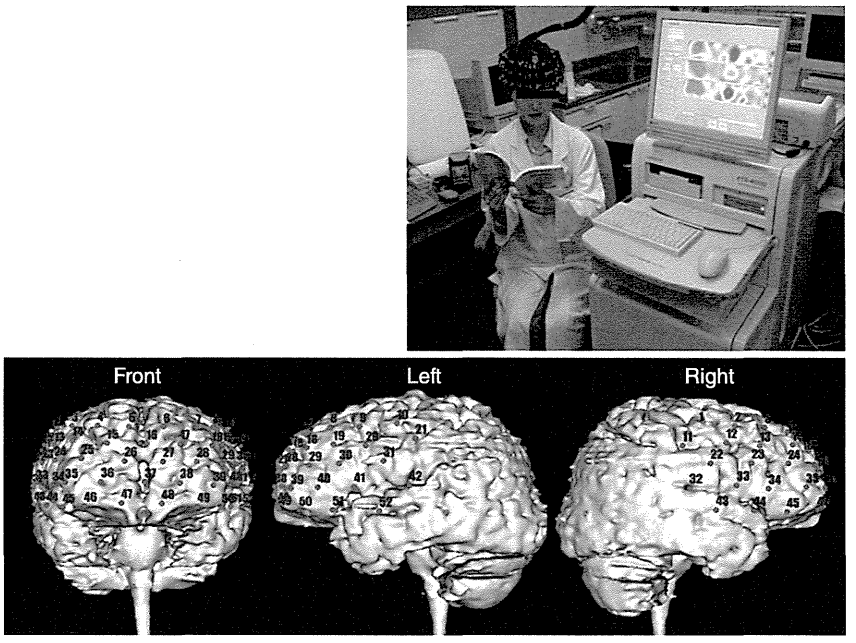

Figure 1 Near-infrared spectroscopy. An optic fiber device connected to a probe is placed on the subject's scalp covering the frontal to temporal regions. The relative concentration of oxygenated hemoglobin (oxy-Hb) was measured every 0.1 s during word-fluency testing.

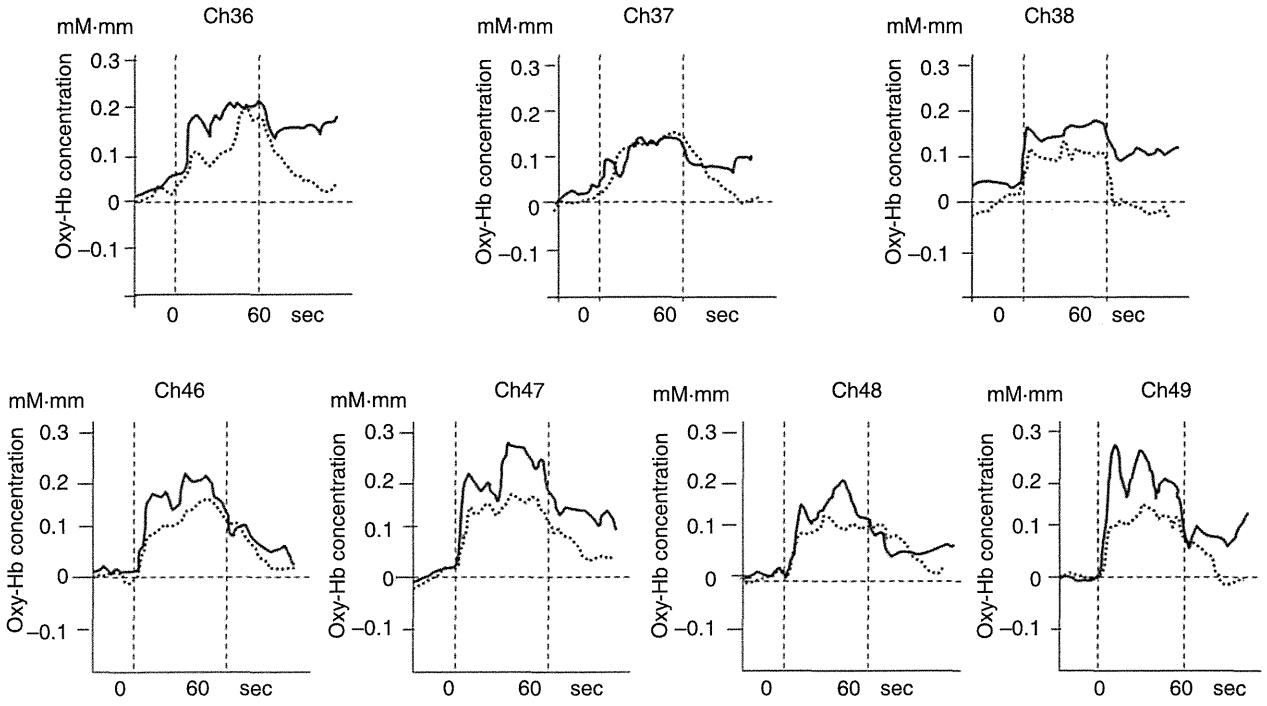

Figure 2 Time-dependent changes in oxygenated hemoglobin (oxy-Hb) concentration in response to tasks. The average waveforms of time-dependent changes in oxy-Hb concentration in representative channels (Ch) are shown. The solid and broken line represents non-minimal hepatic encephalopathy (MHE) and MHE groups, respectively. The area between the two vertical lines corresponds to the 60 s of the word-fluency test.

group, the oxy-Hb concentration increased immediately after the start of the task, remained high with repetitive steep peaks during the task, and decreased after the end of the task. In contrast, the time course of oxy-Hb changes was somewhat different in the MHE group, characterized by a slow increase of oxy-Hb throughout the task, gradually reaching a plateau at the end of the task (Fig. 2). These differences in the degree of oxy-Hb changes also could be visualized by the topographic presentation. In the topographic image, increase of oxy-Hb concentration is expressed as a deepening of the red shading. Figure 3 shows a topographic image showing the increase in oxy-Hb concentration in response to a task. The image in Figure 3 is the average value (arithmetic mean topographic image) of all patients. The concentration of oxy-Hb is small in the MHE group, as reflected by blue or green color, compared to the non-MHE group, as reflected by orange or red color.

When the average value of the seven channels were calculated, the maximum value of oxy-Hb increase was smaller in MHE compared to non-MHE patients but it did not reach statistical significance (0.26 ± 0.12 vs 0.32 ± 0.22 mM·mm, $P = 0.37$) (Fig. 4). On the other hand, increase in oxy-Hb concentration at 5 s after starting the task was significantly small in MHE compared to non-MHE patients (0.03 ± 0.05 vs

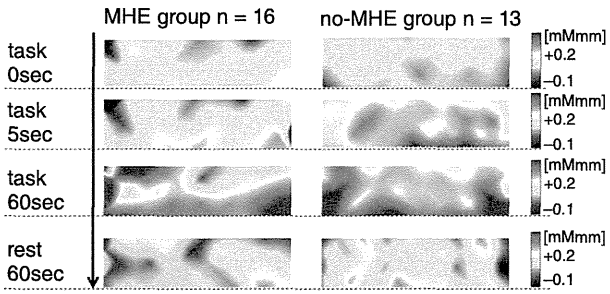

Figure 3 Topographic image showing cumulative increase in oxygenated hemoglobin (oxy-Hb) concentration. Increase in oxy-Hb concentration is shown by deepening of the red shading. The concentration of oxy-Hb is small in the minimal hepatic encephalopathy (MHE) group, as reflected by the blue or green color compared to the non-MHE group as reflected by orange or red color.

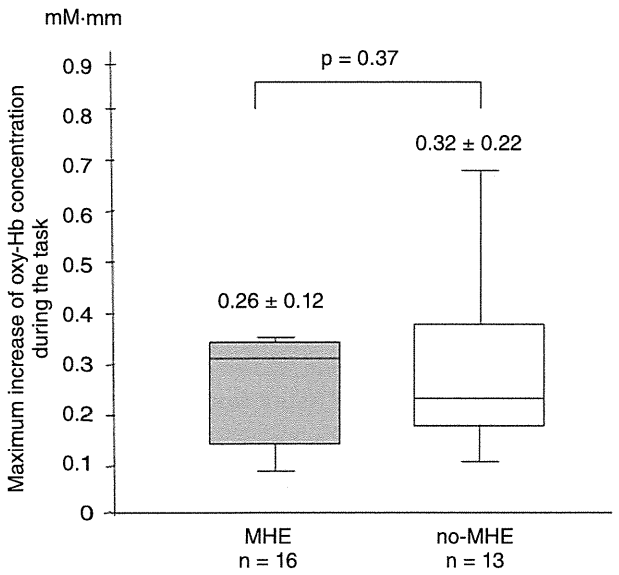

Figure 4 Comparison of maximum increase in oxygenated hemoglobin (oxy-Hb) concentration between patients with and without minimal hepatic encephalopathy (MHE). The average value of maximum increase in oxy-Hb did not differ significantly between the MHE and non-MHE groups.

0.11 ± 0.09 mM · mm, $P = 0.006$) (Fig. 5). For the diagnosis of MHE, the receiver–operator curve analysis identified an optimal cut-off of 0.05 mM · mm for the oxy-Hb concentration at 5 s after starting the task. The area under the curve was 0.774 ($P = 0.012$; 95% confidence interval, 0.60–0.95), sensitivity and specificity of NIRS for the diagnosis of MHE was 69% and 77%, respectively. The positive predictive value was 79% and negative predictive value was 67%.

DISCUSSION

USING NIRS, WHICH can detect changes in regional cerebral oxy-Hb concentration with an extremely high level of sensitivity, we found that increase in cerebral oxy-Hb concentration in response to tasks was slow and small among cirrhotic patients without OHE but having abnormal electroencephalography findings. The impairment of response was most significant at an early time point after the start of the task. These findings indicated that cerebral oxygen metabolism is poorly reactive in response to tasks among patients with MHE and that this impaired cerebral oxygen metabolism may be related to the pathogenesis of latent impairment of brain activity seen in MHE. To the best of our knowledge, our study appears to be the first evaluating MHE with NIRS. The non-invasiveness and high time resolution of NIRS give it potential as a valuable research tool for the examination of brain function in HE, as well as a clinically useful tool for the diagnosis of MHE.

Hepatic encephalopathy in its early stage, such as latent or minimal HE, can reduce cognitive function, lower work efficiency, reduce QOL[27,28] or impair driving skill.[1,2,29,30] Although there are several practical requirements for the diagnosis of MHE, adequate diagnosis of MHE is difficult due to the lack of reliable diagnostic standards.[31,32] Several diagnostic methods such as neuropsychological function tests, number connection test, light/sound reaction time, inhibitory control test, WAIS or electro-psychological tests including EEG, spectral EEG, and cerebral evoked potential, PHES, critical flicker test and computer-aided quantitative neuropsychological function test system (NP-test)[7–15] have been proposed,[32–36] but there is no ideal test for MHE as yet. Because these tests are developed for the screening of MHE, these are not diagnostic. Establishment of a reliable diagnostic method for MHE is imperative. We

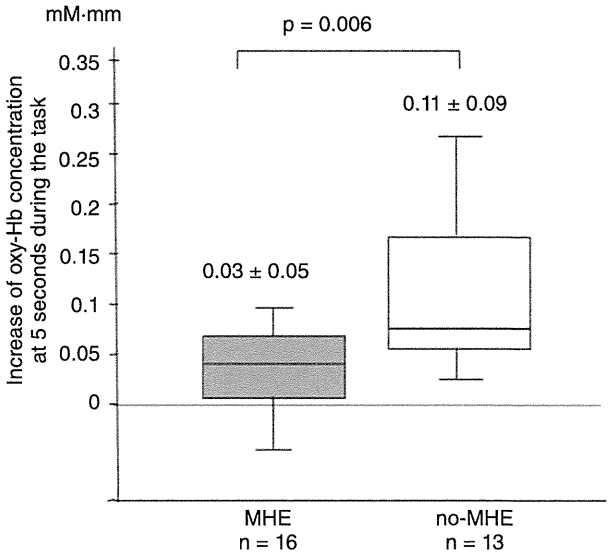

Figure 5 Comparison of increase in oxygenated hemoglobin (oxy-Hb) concentration at 5 s after the start of task between patients with and without minimal hepatic encephalopathy (MHE). The average value of increase in oxy-Hb was compared between the MHE and non-MHE groups at 5 s after starting the word-fluency task. The increase in the oxy-Hb concentration was significantly lower in patients with MHE compared to non-MHE ($P = 0.006$).

have some cases in which NIRS results improved with lactulose and branched-chain amino acid. A prospective study is ongoing to evaluate the effect of treatment by NIRS. The major advantage of NIRS over "paper and pencil tests" is the absence of learning effect which is generally seen in other neuropsychological function tests[37] and NIRS could also discriminate other mental disorders.[24,25]

Neuroimaging using MRI, magnetic resonance spectroscopy and PET has made it possible to non-invasively assess hepatic encephalopathy.[38–47] However, these tests require extensive equipment and are therefore costly. NIRS is a new methodology for brain research and brain function testing, and has applications in various areas of medicine, being used not only in research, but also in clinical medicine.[23–25,48] NIRS has been approved for identifying the language-dominant hemisphere before brain surgery and measuring epileptic foci.[49] In human studies comparing NIRS and fMRI,[50–52] a correlation was seen between blood-oxygen-level-dependent signal and oxy-Hb concentration as measured by NIRS. In brain function analysis, the detection sensitivity of NIRS is comparable to that of fMRI, but the time resolution of NIRS is greater. Furthermore, the advantages of NIRS are convenience, bedside analysis, non-invasiveness, free task setting and low cost.

Here, we used multichannel NIRS to measure the changes in oxy-Hb concentration during task performance from the frontal to temporal regions of the cortex in MHE patients and compared the results with those of liver cirrhosis without MHE. In all subjects, oxy-Hb increased during task performance and gradually decreased after the completion of task performance. However, the time-dependent changes in the degree of increase in oxy-Hb concentration differed between patients with and without MHE. The degree of increase in oxy-Hb concentration during task performance was smaller and more gradual in MHE compared to non-MHE patients. The increase of the oxy-Hb concentration reflects the increase of cerebral blood volume in the area of the brain activated by the task. Iversen *et al.* found that the cerebral oxygen consumption and blood flow were both reduced in cirrhotic patients with an acute episode of OHE[16]and that the oxygen delivery was approximately twice the oxygen consumption, indicating that oxygen delivery or blood flow was not a limiting factor for the oxygen consumption. Consequently, cerebral blood flow seems to be reduced as a result of diminished cerebral oxygen requirement during HE, and not vice versa.[16] It is reported that neuron-to-astrocyte signaling is a key mechanism in functional hyperemia,[17–19,53,54] and that function of astrocytes is impaired in hepatic encephalopathy patients.[20–22] Therefore, impaired astrocyte-mediated control of cerebral microcirculation can result in slow increase of cerebral blood flow during task performance in MHE patients. Thus, the sluggish increase in cerebral blood flow seen in MHE in the present study may reflect the impaired brain activity and dysfunction of astrocytes and impaired cerebral oxygen metabolism in these patients.

There are several limitations in the present study. The number of patients was not enough to make a comparison stratified by Child grade. We would like to analyze this important point in a future study. It may be possible that cerebral oxy-Hb may change due to aging or by the arteriosclerotic changes. In the present study, age was not related to NIRS results. All patients were examined by brain MRI or brain CT and they had no apparent brain structural disease including brain infarction. However, it was not possible to evaluate the arteriosclerotic changes. This may be another limitation of this study. Many neuropsychological function tests, such as number connection test, light/sound reaction time, inhibitory control test, WAIS or electro-psychological tests including EEG, cerebral evoked potential, p300 event-related potential, PHES and critical flicker test have been employed for the diagnosis of MHE. In Japan, Kato and colleagues established the computer-aided quantitative neuropsychological function test system called NP-test.[7] However, these tests were not simultaneously measured in the present study. Because we recognize the importance of comparing NIRS with other tests, we would like to solve this issue in future study.

In conclusion, NIRS, with its high degree of time resolution, enabled us to identify the characteristic time course of oxy-Hb concentration changes during tasks in MHE. The observations imply that cerebral oxygen supply and metabolism is poorly reactive in MHE, which may be related to the pathogenesis of latent impairment of brain activity.

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Hepatology Research

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Original Article

Clinical efficacy of combination therapy with ME3738 and pegylated interferon-alpha-2a in patients with hepatitis C virus genotype 1

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Aim: ME3738, a derivative of soyasapogenol B, enhances the anti-hepatitis C virus (HCV) effect of interferon in an *in vitro* replication system and an *in vivo* mouse model of HCV infection. ME3738 plus pegylated interferon (PEG IFN)-α-2a treatment for 12 weeks decreased HCV RNA levels in enrolled late virus responder (LVR) patients with relapsed HCV. Half of the patients reached undetectable HCV RNA level. The present clinical study of ME3738 was conducted in naïve chronic hepatitis C patients to investigate the sustained virological response (SVR) and safety of 48-week treatment with ME3738 plus PEG IFN-α-2a.

Methods: Subjects (n = 135) with genotype 1b chronic hepatitis C with high viral loads were divided into three groups (ME3738 50 mg b.i.d., 200 mg b.i.d. or 800 mg b.i.d.). ME3738 was administrated p.o. and PEG IFN-α-2a (180 μg/week) s.c. for 48 weeks, and SVR was assessed at 24 weeks of treatment-free follow up.

Results: The viral disappearance rates at 12 and 48 weeks were 23.0% and 48.9%, respectively. SVR was seen in 5.9% of subjects. ME3738 did not worsen the adverse reactions generally seen with PEG IFN-α-2a treatment, and any adverse reactions specific to ME3738 were not observed.

Conclusion: ME3738 plus PEG IFN-α-2a treatment to naïve chronic hepatitis C patients showed an antiviral effect and a good safety profile up to 48 weeks. However, HCV RNA was again detected in many subjects after treatment termination. Even though ME3738 is not enough to suppress HCV reproduction in this treatment. ME3738 was concurrently used with PEG IFN-α-2a treatment; however, a clear additional effect on SVR was not confirmed.

Key words: clinical efficacy, hepatitis C virus, ME3738, pegylated interferon-alpha-2a

INTRODUCTION

HEPATITIS C VIRUS (HCV) causes chronic hepatitis through persistent infection and is a major cause of liver cancer, particularly in Japan.[1,2] The standard therapy for chronic hepatitis C is co-administration of pegylated interferon (PEG IFN) and ribavirin (RBV), with a reported efficacy rate of approximately 50% in refractory cases with genotype 1b and high levels of plasma HCV RNA.[3] In Japan, many patients with chronic hepatitis C are elderly at present, and this standard therapy in these patients can result in unavoidable treatment discontinuation, low therapeutic effect due to a high incidence of adverse reactions, the need for dose reduction of PEG IFN because of decreased neutrophil count, or the need for dose reduction of RBV because of anemia.[4,5]

ME3738 is a derivative of soyasapogenol B derived from soybeans and ME3738 has been shown to

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suppress liver inflammation in various hepatitis mouse models.[6,7] In patients with chronic hepatitis C with elevated alanine aminotransferase (ALT) values, ME3738 monotherapy showed a tendency toward suppression of liver inflammation, and the only major adverse reactions were digestive symptoms and abnormal laboratory data such as 10% of the patients enrolled in frequency.

ME3738 has also shown to enhance the suppressive effect of IFN on HCV in an *in vitro* replication system of HCV and in an *in vivo* HCV-infected mouse model (chimeric mice).[8,9]

A clinical study of ME3738 was conducted in patients with chronic hepatitis C to confirm viral disappearance with 12 weeks of treatment.[10] Patients were those with chronic hepatitis C who had received combination therapy with PEG IFN-α-2b and RBV. Among the patients who achieved viral disappearance after 12–24 weeks of treatment (late virus responders: LVR), those who showed viral reactivation after 48 weeks of treatment were enrolled.

ME3738 was administrated p.o. twice a day at a dose of 50 mg/day or 800 mg/day, and PEG IFN-α-2a was administrated s.c. at a dose of 180 μg once a week. This combination therapy was administrated for 12 weeks. In all subjects enrolled in this study, viral disappearance had not been achieved by previous therapy (co-administration of PEG IFN-α-2b and RBV) within 12 weeks of treatment (HCV RNA, ≥1.7 log IU/mL). On the other hand, when ME3738 was co-administrated, viral disappearance was achieved in approximately 50% of subjects within 12 weeks of treatment. Therefore, it was suggested that ME3738 co-administrated with PEG IFN would contribute to early viral disappearance.

The present clinical study of ME3738 was conducted in naïve chronic hepatitis C patients to investigate the sustained virological response (SVR) and safety of 48-week treatment with ME3738 plus PEG IFN-α-2a.

METHODS

Study design and dosing

THIS STUDY WAS conducted as a multicenter, randomized, single-blind, comparative phase II study (protocol no. ME3738-11).

ME3738 used in the study was provided by Meiji Seika Pharma (Tokyo, Japan).

ME3738 doses were selected as 50 mg/day and 800 mg/day, the safety of which was confirmed in a previous study using 12-week combined administration. In addition, 200 mg/day was selected as an intermediate dose to confirm a dose-dependent effect on SVR, and a total of three doses were selected.

Subjects were divided into three groups, with ME3738 administrated p.o. twice a day (after breakfast and dinner) at a dose of 50 mg/day, 200 mg/day or 800 mg/day. PEG IFN-α-2a 180 μg was administrated s.c. to all subjects once a week. This combination therapy was administrated for 48 weeks. Before starting treatment, subjects were assigned to each of the three groups at the central registration center, so that the number of subjects in each group was identical.

Patients were assigned doses based on an allocation chart that was preliminarily prepared in a central registration center (Moss Institute), which was a party independent of the investigative group.

In subjects who achieved disappearance of HCV RNA at the completion of the 48-week treatment (end of treatment: EOT), follow-up observation was performed for an additional 24 weeks (24-week follow-up after EOT) to confirm SVR.

During the study period, blood cell counts were measured every week before administration of PEG IFN-α-2a, and the dose of PEG IFN-α-2a was reduced to 90 μg or withdrawn according to the following criteria:

- Dose reduction: neutrophil count less than 750/mm^3 or platelet count less than 50 000/mm^3
- Dose withdrawal (suspension): neutrophil count less than 500/mm^3, platelet count less than 25 000/mm^3 or hemoglobin less than 8.5 g/dL.

The following study discontinuation criteria were set. When the HCV RNA determined after 12 weeks of treatment was not lower than the baseline level by at least 2.0 Log IU/mL, the study was to be discontinued in the subject concerned. When HCV RNA was detected after 48 weeks of treatment or during the follow-up period, the study was also to be discontinued in the subject concerned at the time point of detection.

Subjects

The study population included patients with naïve chronic hepatitis C.

The target patients were aged 20 years or more, with HCV genotype 1b and a high viral load (≥5.0 log IU/mL).

Patients meeting the following conditions were excluded from this study: patients positive for hepatitis B surface antigen, patients with hepatitis other than chronic hepatitis C, patients with liver cirrhosis or liver cancer, patients with a FibroIndex[11] not less than 2.25 (F4), and patients with a baseline neutrophil count less than 1500/mm^3, a baseline platelet count less

than 90 000/mm^3, or a baseline hemoglobin value less than 10 g/dL. Patients with a complication or anamnesis that could possibly influence the safety or efficacy of ME3738 were also excluded. Concomitant drugs or therapies that could possibly influence the safety and efficacy of ME3738, such as systemic antiviral drug and double-membrane filtration plasmapheresis, were also prohibited during the study period.

This study was conducted in accordance with Good Clinical Practice guidelines, conforming to the Declaration of Helsinki. Written informed consent was obtained from all patients before their enrollment in this study, which was conducted with the approval of the institutional review board of each study site.

Safety and efficacy assessments

Assessments were performed at baseline before starting the study; after 2, 4, 8 and 12 weeks of treatment; every 4 weeks thereafter; at EOT; and during the follow-up period. Assessments included inquiries, vital sign measurements, laboratory tests, adverse event surveys and HCV RNA determinations.

When any adverse event was noted, follow-up examinations were performed in the subject concerned and, if necessary, after study completion.

The amount of HCV RNA during the study period was determined by the TaqMan polymerase chain reaction (PCR) method.

At each determination time point, the disappearance of HCV RNA was judged when no signal was detected with the TaqMan-PCR method. Efficacy was assessed as the HCV disappearance rate after 12 weeks of treatment and at week 24 of the follow-up period. When "HCV RNA undetectability" was maintained until week 24 of the follow-up period, SVR was judged in the subject concerned.

The 16 subjects who participated in the extended treatment study (study ME3738-12) after receiving the treatment in this study were tabulated as subjects without SVR.

Mutations of the HCV core protein (amino acid [a.a.]70 and a.a.91), mutations of the IFN-sensitivity determining region (ISDR) and single nucleotide polymorphisms (SNP) of the *IL28B* gene (five sites: rs8103142, rs11881222, rs8099917, rs12980275 and rs12979860) were determined as background factors of the subjects.

Statistical analysis

Background factors were tabulated by each treatment group, and the intergroup bias was assessed using Student's *t*-test or one-way ANOVA for continuance data and Fisher's exact test for categorical data. A two-sided significance level of 5% was adopted.

In the efficacy evaluation, the rate of subjects with SVR was calculated for each treatment group, and the intergroup difference and the 95% confidence interval was determined. In the safety evaluation, adverse events and adverse reactions were tabulated for each treatment group to determine the incidence rate. The Medical Dictionary for Regulatory Activities (MedDRA) ver. 14.0 was used for tabulation of adverse events.

RESULTS

OF THE 135 subjects (male, 49; female, 86) included in the study, 46 were in the ME3738 50-mg/day group, 45 in the ME3738 200-mg/day group and 44 in the ME3738 800-mg/day group. The mean age was 57.6 years (range, 23–76) with elderly subjects over 60 years occupying more than half of the study population. As for sex, elderly women were dominant.

As per the protocol, 24 subjects in whom the viral amount did not decrease from the baseline level by at least 2 log IU/mL after 12 weeks of treatment were discontinued from the study. An additional three subjects were discontinued for other reasons, resulting in a total of 27 subjects being withdrawn from the study. As a result, study treatment was continued in 108 subjects after 12 weeks of treatment.

Of these 108 subjects, 16 subjects were discontinued from the study for various reasons, including occurrence of an adverse event. A total of 92 subjects completed the 48-week treatment. Among the subjects in whom HCV RNA was positive after 12 weeks of treatment and negative conversion was achieved with 36 weeks of treatment (LVR), 16 subjects from whom consent was obtained participated in the extended treatment study (study ME3738-12) and received 24 weeks of extended treatment in continuation from the 48-week treatment in this study.

Therefore, the follow-up observation was performed in 47 subjects who achieved viral disappearance after 48 weeks of treatment and did not participate in the extended treatment study, and SVR was judged in eight of those subjects after 24 weeks of follow-up observation (Fig. 1).

The major patient background factors showed no biases among the three treatment groups (ME3738 50-mg/day, ME3738 200-mg/day and ME3738 800-mg/day groups) (Table 1). SNP of the *IL28B* gene were determined in 126 subjects who provided consent for

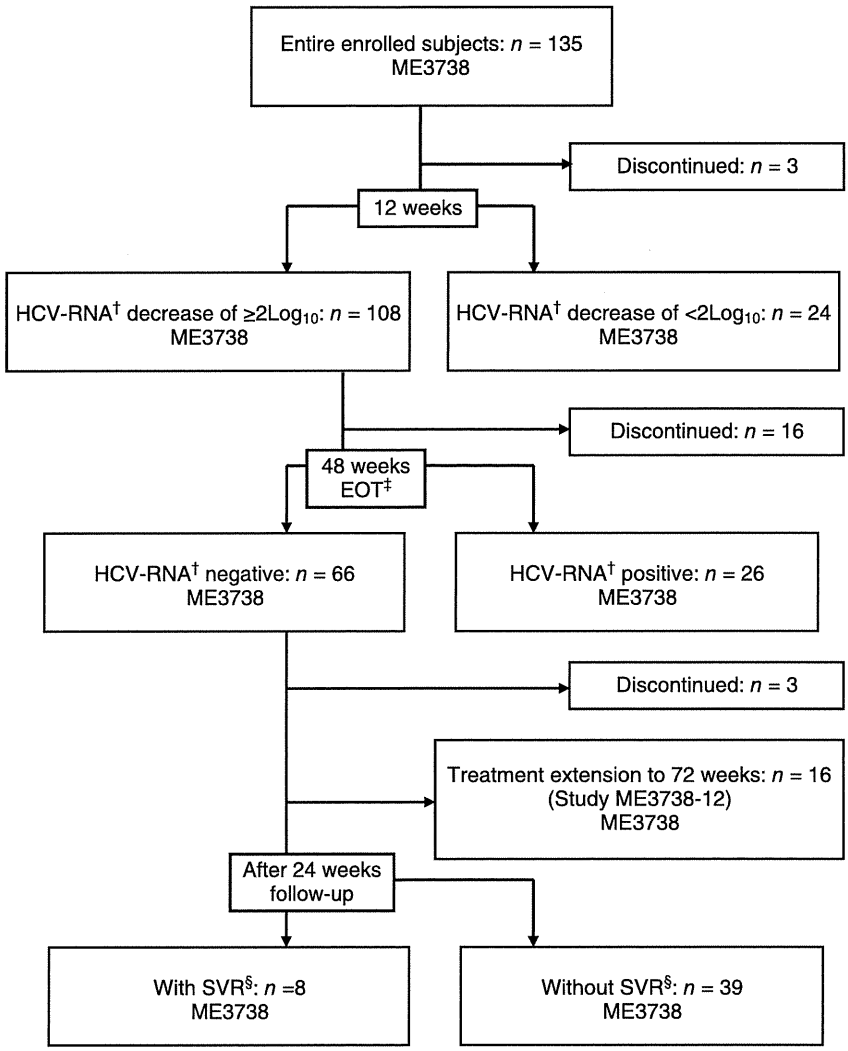

Figure 1 Subject disposition. †Hepatitis C virus. ‡End of treatment. §Sustained virological response.

the analysis. Among the 126 subjects, the result for rs12980275 was different from the results of the other four SNP in three subjects, whereas the results of all five SNP were coincident in the remaining 123 subjects. Even in the three subjects in whom the result for rs12980275 was different, the results for the other four SNP were coincident, allowing the coincident results of four SNP to be included in the tabulation.

The SNP of *IL28B* determined in this study were a major homo allele in 68.1% (92/126) of the subjects, a minor hetero allele in 24.4% (33/126) of the subjects and a minor homo allele in 0.7% (1/126) of the subjects. These rates were similar to the results previously reported in Japan.[11]

Efficacy

Table 2 shows the virological effects at each stage of this study. The viral disappearance rate indicating the proportion of subjects who achieved viral disappearance was 5.9% (8/135) after 4 weeks of treatment, 23.0% (31/135) after 12 weeks of treatment and 48.9% (66/135) at EOT. The rate of subjects with SVR was 5.9% (8/135) after 24 weeks of follow-up observation. The viral reactivation rate, meaning the proportion of subjects in whom viral regrowth was observed during the follow-up observation period from among the total subjects included in the follow-up observation, was 83.0% (39/47), and reactivation occurred within 12 weeks of

Table 1 Characteristics of study patients

		PEG IFN-α-2a§ + ME3738 50 mg/day ($n = 46$)	PEG IFN-α-2a§ + ME3738 200 mg/day ($n = 45$)	PEG IFN-α-2a§ + ME3738 800 mg/day ($n = 44$)	Total ($n = 135$)	Test result
Sex	Male	17 (37.0%)	16 (35.6%)	16 (36.4%)	49 (36.3%)	$P = 1.0000$ (Fisher's exact test)
	Female	29 (63.0%)	29 (64.4%)	28 (63.6%)	86 (63.7%)	
Age	Mean	56.5	59.8	56.6	57.6	$P = 0.3166$ (1-way ANOVA)
	Standard deviation	12.6	9.8	11.9	11.5	
	Median	61	61	60	60	
	Range	23–73	40–75	26–76	23–76	
Bodyweight	Mean	55.9	55.9	57.6	56.5	$P = 0.7033$ (1-way ANOVA)
	Standard deviation	12.1	11.3	9.8	11.1	
	Median	54.5	54.0	55.6	54.9	
	Range	34.9–88.6	39.2–82.5	43.0–84.0	34.9–88.6	
Latest liver biopsy result	F0–F2	21 (45.7%)	19 (42.2%)	14 (31.8%)	54 (40.0%)	$P = 0.4524$ (Fisher's exact test)
	F3	0 (0.0%)	1 (2.2%)	2 (4.5%)	3 (2.2%)	
	No test result	25 (54.3%)	25 (55.6%)	28 (63.6%)	78 (57.8%)	
Presence/absence of complication	Absence	2 (4.3%)	6 (13.3%)	5 (11.4%)	13 (9.6%)	$P = 0.3167$ (Fisher's exact test)
	Presence	44 (95.7%)	39 (86.7%)	39 (88.6%)	122 (90.4%)	
Presence/absence of diabetes	Absence	43 (93.5%)	45 (100.0%)	42 (95.5%)	130 (96.3%)	$P = 0.2843$ (Fisher's exact test)
	Presence	3 (6.5%)	0 (0.0%)	2 (4.5%)	5 (3.7%)	
Presence/absence of concomitant drug	Absence	0 (0.0%)	0 (0.0%)	1 (2.3%)	1 (0.7%)	$P = 0.3259$ (Fisher's exact test)
	Presence	46 (100.0%)	45 (100.0%)	43 (97.7%)	134 (99.3%)	
Presence/absence of concomitant therapy	Absence	29 (63.0%)	32 (71.1%)	35 (79.5%)	96 (71.1%)	$P = 0.2356$ (Fisher's exact test)
	Presence	17 (37.0%)	13 (28.9%)	9 (20.5%)	39 (28.9%)	
Baseline hemoglobin	Mean	13.9	14.2	14	14	$P = 0.6521$ (1-way ANOVA)
Pegasys dose change	No	26 (56.5%)	29 (64.4%)	25 (56.8%)	80 (59.3%)	$P = 0.8599$ (Fisher's exact test)
	Dose reduction	13 (28.3%)	12 (26.7%)	12 (27.3%)	37 (27.4%)	
	Dose withdrawal	7 (15.2%)	4 (8.9%)	7 (15.9%)	18 (13.3%)	
HCV RNA-1b† (NS5A)	0 or 1	45 (97.8%)	39 (86.7%)	40 (90.9%)	124 (91.9%)	$P = 0.1333$ (Fisher's exact test)
	Not less than 2	1 (2.2%)	6 (13.3%)	4 (9.1%)	11 (8.1%)	
HCV RNA-1b† IFN/RBV mutation at 70	Wild type	37 (80.4%)	26 (57.8%)	29 (65.9%)	92 (68.1%)	$P = 0.0611$ (Fisher's exact test)
	Mutant type/ competitive type	9 (19.6%)	19 (42.2%)	14 (31.8%)	42 (31.1%)	
	Other	0 (0.0%)	0 (0.0%)	1 (2.3%)	1 (0.7%)	
HCV RNA-1b† IFN/RBV‡ mutation at 91	Wild type	34 (73.9%)	23 (51.1%)	31 (70.5%)	88 (65.2%)	$P = 0.0529$ (Fisher's exact test)
	Mutant type/ competitive type	12 (26.1%)	22 (48.9%)	13 (29.5%)	47 (34.8%)	
Baseline HCV RNA†	Mean	6.7	6.6	6.7	6.6	$P = 0.4157$ (1-way ANOVA)
FibroIndex	Mean	1.43	1.5	1.41	1.45	$P = 0.5513$ (1-way ANOVA)
IL-28B	Not determined	5 (10.9%)	1 (2.2%)	3 (6.8%)	9 (6.7%)	$P = 0.8000$ (Fisher's exact test)
	Major homo allele	31 (67.4%)	32 (71.1%)	29 (65.9%)	92 (68.1%)	
	Minor hetero allele	9 (19.6%)	12 (26.7%)	12 (27.3%)	33 (24.4%)	
	Minor homo allele	1 (2.2%)	0 (0.0%)	0 (0.0%)	1 (0.7%)	

†Hepatitis C virus.
‡Interferon/ribavirin.
§Pegylated interferon alpha-2a.

Table 2 Undetectable HCV RNA during the treatment period

No. of subjects (%)		Treatment period						
		4 weeks	8 weeks	12 weeks	24 weeks	48 weeks (EOT†)	Follow up‡ (12 weeks)	Follow up‡ (24 weeks)
PEG IFN-α-2a§ + ME3738 50 mg/day	$n = 46$	2	8	12	23	23	3	3
		4.3%	17.4%	26.1%	50.0%	50.0%	6.5%	6.5%
PEG IFN-α-2a§ + ME3738 200 mg/day	$n = 45$	3	4	12	20	26	5	3
		6.7%	8.9%	26.7%	44.4%	57.8%	11.1%	6.7%
PEG IFN-α-2a§ + ME3738 800 mg/day	$n = 44$	3	5	7	12	17	3	2
		6.8%	11.4%	15.9%	27.3%	38.6%	6.8%	4.5%
Total	$n = 135$	8	17	31	55	66	11	8
		5.9%	12.6%	23.0%	40.7%	48.9%	8.1%	5.9%

†End of treatment.
‡12/24 weeks after EOT.
§Pegylated interferon-α-2a.

follow-up observation in most of the subjects who showed viral reactivation.

Table 3 shows the patient background factors which might, and are considered to, have influenced the SVR in this study. With respect to sex, the effect was higher in men than in women, and with respect to age, the effect was lower in the elderly (≥60 years) than in younger subjects (<60 years). Thus, the effect was the lowest in elderly female subjects. There were no definite differences in FibroIndex showing the grade of liver fibrosis. In terms of ISDR, which is a predictive viral factor for IFN therapy in patients infected with HCV genotype 1b, the effect was high in mutants (no. of mutations,: ≥2), but no clear results were obtained in mutations of Core 70 and Core 91. Finally, in terms of *IL28B* SNP, SVR was only seen with the major homo allele.

Treatment with ME3738 showed no clear influence on ALT levels in this study (no relevant data are shown).

Safety

At least one adverse event was noted to 134 subjects among 135 subjects, excluding one subject in whom the study was discontinued after 5 weeks of treatment. Table 4 shows the adverse events noted with an incidence of at least 20% in the entire study population.

Frequently observed adverse events were fever, malaise, headache, nasopharyngitis, pruritus, retinopathy and diarrhea. Frequently observed abnormal laboratory findings were decreased white blood cell count, decreased platelet count, decreased neutrophil count, increased hyaluronic acid, decreased hemoglobin, decreased hematocrit and decreased red blood cell count.

In this study, decreased hemoglobin was observed in 45.9% (62/135) of subjects, and the hemoglobin level decreased to less than 10 g/dL in the treatment period in 19.3% (26/135) of subjects. Figure 2 shows the time-course of changes in the hemoglobin level in each ME3738 treatment group.

DISCUSSION

IN THIS STUDY conducted in patients with naïve HCV, 135 subjects were administrated ME3738 and 27 subjects were withdrawn from this study by week 12 for various reasons, including applicability to the study discontinuation criteria specified in the protocol. As a result, study treatment was continued in 108 subjects after 12 weeks of treatment. Among these, 16 subjects discontinued the study for reasons such as occurrence of an adverse event. There were 92 subjects who completed

Table 3 Comparison of clinical characteristics and viral types between subjects with and without sustained virological response

	Virological response	
	Subjects with SVR†, *n* (%)	Subjects without SVR†, *n* (%)
SVR†		
Entire subjects	8/135 (5.9%)	127/135 (94.1%)
By sex and age		
Men	5/49 (10.2%)	44/49 (89.8%)
Age, <60 years	4/23 (17.4%)	19/23 (82.6%)
Age, ≥60 years	1/26 (3.8%)	25/26 (96.2%)
Women	3/86 (3.5%)	83/86 (96.5%)
Age, <60 years	3/36 (8.3%)	33/36 (91.7%)
Age, ≥60 years	0/50 (0%)	50/50 (100%)
FibroIndex		
>1.25	5/95 (5.3%)	90/95 (94.7%)
≤1.25	3/40 (7.5%)	37/40 (92.5%)
ISDR‡		
Wild (0–1)	5/124 (4.0%)	119/124 (96.0%)
Mutant (>2)	3/11 (27.3%)	8/11 (72.7%)
Core region amino acid substitution site		
Wild	5/92 (5.4%)	87/92 (94.6%)
70-mutant	3/43 (7.0%)	40/43 (93.0%)
Wild	2/88 (2.3%)	86/88 (97.7%)
91-mutant	6/47 (12.8%)	41/47 (87.2%)
Genotyping of *IL-28B* SNP§		
Major homo allele	8/92 (8.7%)	84/92 (91.3%)
Minor hetero/homo allele	0/34 (0%)	34/34 (100%)

†Sustained virological response.
‡Interferon sensitivity determining region.
§Single nucleotide polymorphism.

the 48-week treatment. As compared with the standard combination therapy of PEG IFN-α-2b and RBV, it appeared that the subject withdrawal rate was lower and the treatment completion rate was higher in the current study.

An antiviral effect was seen in 48.9% of subjects after 48 weeks of treatment, but SVR was judged in only 5.9% of subjects at the end of the follow-up observation period. On the other hand, in a clinical study of combination therapy with PEG IFN-α-2a plus RBV conducted in Japan in patients with naïve chronic hepatitis C (HCV genotype 1b, high viral load), the viral disappearance rate was 86.9% (86/99) and SVR was judged in 59.4% (57/96) of subjects at EOT.[12] In the combination therapy with ME3738 plus PEG IFN-α-2a, the proportion of subjects with SVR was markedly lower than that of subjects who achieved viral disappearance after 12 or 48 weeks of treatment, suggesting that suppression of viral reactivation is weaker with the combination therapy that includes ME3738 than with the combination therapy that includes RBV.

In addition, there were no differences in SVR among the three different ME3738 doses.

In terms of the influence of ME3738 treatment on ALT levels, no consistent tendency could be found in the combination therapy used in this study.

In the safety evaluation, most adverse events noted in this study were comparable in severity and frequency with the events frequently noted with PEG IFN-α-2a monotherapy,[13] and no new adverse events were noted with the ME3738 combination therapy, apart from the two events described below. This suggests that the adverse events noted with ME3738 plus PEG IFN-α-2a combination therapy were not substantially different in severity and frequency from the adverse events noted with PEG IFN-α-2a monotherapy, and that the safety of ME3738 combination therapy is high.

Increased hyaluronic acid and increased blood immunoglobulin G were adverse events that were not frequently seen with PEG IFN-α-2a monotherapy, but were frequently seen in this study. Because these parameters are seldom determined in a time-course manner in