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ORIGINAL ARTICLE

Antiviral activity, dose–response relationship, and safety of entecavir following 24-week oral dosing in nucleoside-naive Japanese adult patients with chronic hepatitis B: a randomized, double-blind, phase II clinical trial

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Abstract

Purpose A randomized, double-blind, multicenter study (ETV-047) was conducted to evaluate the dose–response relationship of entecavir and compare its antiviral activity and safety with lamivudine in Japanese patients with chronic hepatitis B (CHB).

Methods One hundred thirty-seven nucleoside-naive adult patients with CHB were randomized to once-daily oral doses of entecavir 0.01, 0.1, or 0.5 mg or lamivudine 100 mg for 24 weeks. The primary efficacy end point used to evaluate the dose–response relationship was mean change from baseline in serum hepatitis B virus (HBV) DNA level at week 22, as determined by polymerase chain reaction assay.

Results Entecavir demonstrated a clear dose–response relationship, with mean change from baseline in serum

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HBV DNA level of −3.11, −4.77, and −5.16 $\log_{10}$ copies/ml with entecavir 0.01, 0.1, and 0.5 mg, respectively. Entecavir 0.5 mg was superior to lamivudine 100 mg for the mean change in HBV DNA level (−5.16 vs. −4.29 $\log_{10}$ copies/ml; $P = 0.007$). The overall incidence of adverse events was comparable between treatment groups. Two patients discontinued treatment because of adverse events (one with liver cirrhosis [entecavir 0.5 mg] and one with grade 4 serum alanine aminotransferase (ALT) elevation, nausea, and malaise [lamivudine 100 mg]). Serum ALT flares were observed in four patients; flares were associated with 2 $\log_{10}$ reductions or more in HBV DNA level and resolved without dose interruption.

Conclusion Entecavir 0.01–0.5 mg is well tolerated and produces a dose-dependent reduction in viral load in nucleoside-naive Japanese patients with CHB. Compared with lamivudine 100 mg, entecavir 0.1 mg demonstrated noninferiority and entecavir 0.5 mg was superior in this population.

Keywords Chronic hepatitis B · Entecavir · Lamivudine · HBV DNA · ALT flare

Introduction

It is reported that more than 2 billion individuals worldwide have been infected with hepatitis B virus (HBV) and approximately 350 million people are long-term HBV carriers [1]. Chronic hepatitis B (CHB) is induced by chronic replication of HBV in the liver and has a poor prognosis, with 20–40% of infected individuals developing liver cirrhosis, noncompensated liver disorder, or hepatocellular carcinoma [2]. Treatment of CHB is aimed at sustained inhibition of HBV replication and remission of liver disease [3], ultimately preventing progression to liver cirrhosis or hepatocellular carcinoma [4].

Prior to the advent of the nucleoside analog lamivudine, interferon-α formed the mainstay of treatment, but this immunoregulatory cytokine requires parenteral administration and is poorly tolerated [5]. Lamivudine is well tolerated on oral administration and has been proven to be highly effective in the treatment of CHB, but the emergence of resistance mutations (including the YMDD motif) in the reverse-transcriptase domain of HBV polymerase frequently results in overt viral rebound and disease progression [6–9]. The novel nucleoside analog adefovir is effective against wild-type HBV and lamivudine-resistant strains and is well tolerated on long-term administration, but its clinical use is restricted by the need for renal monitoring in patients with impaired renal function [10].

Entecavir, a cyclopentylguanine-derived nucleoside analog and selective inhibitor of HBV replication, was approved by the U.S. Food and Drug Administration in 2005 for the treatment of CHB. Entecavir displays potent antiviral activity in the woodchuck and duck models of HBV infection [11, 12] and is reported to be 100- to 2,200-fold more potent than lamivudine and adefovir in inhibiting HBV replication in vitro [13, 14]. Phase II clinical trials of entecavir conducted in non-Japanese patients with CHB have demonstrated entecavir to be well tolerated and more effective than lamivudine [15, 16].

A global dose-finding study (ETV-005) conducted in lamivudine-naive patients with CHB compared three doses of entecavir (0.01, 0.1, and 0.5 mg once daily) with lamivudine 100 mg once daily over a 22-week treatment period. Entecavir showed a clear dose–response relationship and was well tolerated at all three dose levels; in addition, 0.1 and 0.5 mg of entecavir showed superior antiviral activity compared with 100 mg of lamivudine [15].

Phase I studies of single-dose (0.05–2.5 mg) and multiple-dose (0.1–1.0 mg daily) entecavir conducted in Japan have confirmed the drug's safety in healthy men. As in Caucasian populations, entecavir displayed linear plasma pharmacokinetics over a wide range of doses, including putative therapeutic doses (0.5 and 1.0 mg), in Japanese subjects; there was no evidence of significant ethnic differences in its pharmacokinetics and pharmacodynamics. Similar findings to those obtained in the global phase II clinical trials of entecavir might therefore be expected from corresponding studies conducted in Japanese patients.

To evaluate the dose–response relationship, the antiviral activity and safety of entecavir in Japanese CHB patients, we conducted a 24-week phase II study comparing entecavir (0.01, 0.1, and 0.5 mg daily) to lamivudine (100 mg daily).

Materials and methods

Study design

This randomized, double-blind, double-dummy study was conducted at 38 institutions in Japan from August 2003 to March 2005. Eligible patients comprised 20- to 75-year-old men and women with CHB who fulfilled the following criteria: (i) HBsAg-positive for 24 weeks or more or IgM HBcAb-negative with biopsy-confirmed CHB; (ii) HBeAg-positive or HBeAg-negative for 12 weeks or more; (iii) serum HBV DNA level 40 MEq/ml or more (143 pg/ml) by Quantiplex™ branched DNA hybridization method (bDNA assay) ($\geq$7.6 $\log_{10}$ genome equivalent by the transcription-mediated amplification method or $\geq 10^{7.6}$ copies/ml by Roche Amplicor™ polymerase chain reaction method [PCR assay]) measured 2 weeks or more before screening and serum HBV DNA level 40 MEq/ml or more (by bDNA assay) at screening; (iv) serum alanine

aminotransferase (ALT) level 1.25–10 times the upper limit of normal (ULN); and (v) well-compensated liver disease with prothrombin time prolongation 3 s or less or international normalized ratio 1.5 or less, serum albumin level 3.0 g/dl or more, and total bilirubin 2.5 mg/dl or less (42.75 μmol/l). After a 6-week screening period, eligible patients were stratified according to HBeAg status and study site and randomized (1:1:1:1) to oral treatment with entecavir (0.01, 0.1, or 0.5 mg plus matching placebo capsule) or lamivudine (100 mg plus matching placebo tablet) once daily for 24 weeks. All doses were administered at fixed times of the day, avoiding the 2 h before and after meals. Pregnant women were excluded from the study, as were patients with liver cirrhosis, patients with a history or evidence of variceal bleeding, patients with hepatic encephalopathy or ascites requiring diuretics, or patients with paracentesis. Patients with other liver disease (e.g., autoimmune hepatitis) were excluded from the study. In addition, patients were excluded if they had a serum creatinine level more than 1.5 × ULN, hemoglobin level less than 10.0 g/dl, platelet count less than 70,000/mm^3, granulocyte count less than <1,500/mm^3 or plasma α-fetoprotein level more than 100 ng/ml, a history of allergy induced by nucleoside analog or exposure to nucleoside analogs, a recent history (previous 24 weeks) of treatment with immunosuppressives or interferon-α/β, or current treatment of CHB.

Treatment efficacy was assessed after 22 weeks, and all eligible patients who completed 24 weeks of blinded therapy were given the option of enrolling in a separate entecavir trial. Patients who discontinued therapy prematurely were followed up for 24 weeks postdosing. Patients began anti-HBV therapy as recommended by their physician during the postdosing follow-up period.

Informed consent was obtained from all patients in writing prior to their inclusion in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines and notifications were issued by the Ministry of Health and Labor.

Efficacy and safety assessment

The primary efficacy end point for the evaluation of the dose–response relationship of entecavir was the change from baseline in mean serum HBV DNA level at week 22, as determined by PCR assay. Secondary efficacy end points for the assessment of the noninferiority of entecavir at each dose to lamivudine included the change from baseline in mean serum HBV DNA level at week 22, as determined by PCR assay, the percentage of patients with a reduction in serum HBV DNA level 2 $\log_{10}$ copies/ml or more or a serum HBV DNA level below the limit of detection (400 copies/ml by PCR assay; 2.5 pg/ml or 0.7 MEq/ml by bDNA assay) at week 22, the percentage of patients with HBeAg loss, the percentage of patients with HBeAg seroconversion (HBeAg loss and appearance of HBe-antibody), the percentage of patients achieving ALT normalization (World Health Organization grade 0: <1.25 × ULN), and the percentage of patients achieving a protocol-defined response (HBV DNA level <0.7 MEq/ml by bDNA assay, HBeAg negativity and serum ALT level <1.25 × ULN for HBeAg-positive patients; HBV DNA level <0.7 MEq/ml by bDNA assay and serum ALT level <1.25 ULN for HBeAg-negative patients) at week 22. The incidence of genotypic drug resistance was also assessed in patients who had a 1 $\log_{10}$ copies/ml or more increase in HBV DNA by PCR from nadir while on study drug.

Based on the results of the global dose–response study of entecavir conducted in nucleoside-naive patients (ETV-005 study) [15], noninferiority of entecavir 0.1 or 0.5 mg compared with lamivudine (100 mg) was confirmed if the upper 95% confidence interval (CI) for the difference in mean HBV DNA levels at week 22 was 0.8 $\log_{10}$ copies/ml or less.

Assay methods

Serum HBV DNA level was determined by Roche Amplicor™ PCR assay (Roche Diagnostics K.K., Tokyo, Japan) and Quantiplex™ (Chiron) bDNA assay. Clinical laboratory tests, serum HBV DNA assays, and HBV serology were performed at the central clinical laboratory designated by the trial sponsor. Genotypic analysis of HBV isolates was performed using samples collected from patients on the first day of treatment. Genotypic analysis of HBV DNA polymerase was performed at SRL Inc. (Tokyo, Japan).

Statistical analysis

Numerical data were expressed by descriptive statistics. Serum HBV DNA level, a continuous variable, was analyzed after logarithmic transformation. For treatment group, comparisons of continuous variables, analysis of variance models, incorporating baseline HBV DNA level and HBeAg status as covariates were employed. For intertreatment comparisons of binary data, Cochran–Mantel–Haenszel tests were employed using baseline HBeAg status as a stratification factor. For analysis of dose–response relationships, Student's t test was applied to linear regression plots of serum HBV DNA level against log dose. A two-sided $P < 0.05$ was taken to indicate statistical significance. For analysis of dose–response relationships using efficacy data, a two-sided $P < 0.05/3$ was taken to

indicate statistical significance following Bonferroni adjustment.

Results

Study population and demographic characteristics

A total of 137 patients, including 20- to 73-year-old men and women, met the study eligibility criteria and were randomized to the following treatment groups: entecavir 0.01 mg ($n = 35$), entecavir 0.1 mg ($n = 34$), entecavir 0.5 mg ($n = 34$), and lamivudine 100 mg ($n = 34$). Three patients (two in the entecavir 0.5 mg group and one in the lamivudine 100 mg group) discontinued the study prematurely; the reasons for discontinuation were noncompliance (one patient in the entecavir 0.5 mg group) and adverse events (liver cirrhosis in one patient [entecavir 0.5 mg group] and grade 4 serum ALT elevation with nausea and malaise in one patient [lamivudine 100 mg group]). Accordingly, a total of 134 patients (entecavir 0.01 mg group, 35 patients; entecavir 0.1 mg group, 34 patients; entecavir 0.5 mg group, 32 patients; and lamivudine 100 mg group, 33 patients) completed 24 weeks of treatment and were included in the efficacy assessment.

The four treatment groups were matched with respect to gender, age, body weight, and proportion of HBeAg-positive patients (Table 1). Serum HBV DNA levels by PCR assay (mean ± SD) at baseline were 7.94 ± 0.87, 8.09 ± 1.05, 8.39 ± 0.73, and 7.94 ± 0.83 $\log_{10}$ copies/ml for the entecavir 0.01, 0.1, and 0.5 mg and lamivudine 100 mg groups, respectively. With regard to HBV genotype, 124 patients were genotype C, 6 patients were genotype A, 5 patients were genotype B, and 2 patients were genotype F. All patients were nucleos(t)ide-naive and none had been pretreated with interferon therapy.

Virologic response

Mean changes (from baseline) in serum HBV DNA level at week 22 were −3.11, −4.77, and −5.16 $\log_{10}$ copies/ml with entecavir 0.01, 0.1, and 0.5 mg, respectively (Fig 1; Table 2). Estimated differences in serum HBV DNA levels between the 0.1 and 0.5 mg entecavir groups and the low-dose entecavir group (0.01 mg) were determined after adjustment for baseline level and HBeAg status. Estimated intertreatment group differences (adjusted 95% CI) were −1.61 (−2.20 to −1.02) $\log_{10}$ copies/ml between the entecavir 0.01 and 0.1 mg groups and −1.95 (−2.53 to −1.37) $\log_{10}$ copies/ml between the entecavir 0.5 and 0.01 mg groups; both of these differences were statistically significant ($P < 0.0001$). In contrast, the difference in serum HBV DNA levels between the high-dose (0.5 mg) and medium-dose (0.1 mg) entecavir groups was not statistically significant (estimated difference [adjusted 95% CI] −0.23 [−0.69 to 0.23] $\log_{10}$ copies/ml). Taken together, these results demonstrate the superiority of high- and medium-dose entecavir (0.1 and 0.5 mg) compared with low-dose entecavir (0.01 mg) in terms of viral load reduction (Table 3). Linear regression analyses indicated a

Table 1 Baseline demographics and clinical characteristics of treated subjects

	ETV 0.01 mg ($n = 35$)	ETV 0.1 mg ($n = 34$)	ETV 0.5 mg ($n = 34$)	LVD 100 mg ($n = 34$)
Male, *n* (%)	25 (71.4)	23 (67.6)	23 (67.6)	28 (82.4)
Female, *n* (%)	10 (28.6)	11 (32.4)	11 (32.4)	6 (17.6)
Age (years), mean ± SD	42.0 ± 12.5	40.1 ± 9.8	39.8 ± 10.4	42.3 ± 12.6
Weight (kg), mean ± SD	66.2 ± 12.5	64.6 ± 11.9	65.3 ± 11.1	64.4 ± 9.0
Ethnicity Japanese, *n* (%)	35 (100)	34 (100)	34 (100)	34 (100)
HBV DNA ($\log_{10}$ copies/ml by PCR), mean ± SD	7.94 ± 0.87	8.09 ± 1.05	8.39 ± 0.73	7.94 ± 0.83
HBeAg positive, *n* (%)	30 (85.7)	30 (88.2)	30 (88.2)	31 (91.2)
ALT (IU/l), mean ± SD	150.1 ± 111.8	162.0 ± 127.1	142.4 ± 82.2	185.0 ± 130.8
AST (IU/l), mean ± SD	83.2 ± 40.0	114.3 ± 109.4	81.0 ± 43.0	121.6 ± 85.4
Total bilirubin (mg/dl), mean ± SD	0.65 ± 0.25	0.56 ± 0.15	0.66 ± 0.25	0.71 ± 0.28
HBV genotype (%)				
C	32 (91.4)	30 (88.2)	32 (94.1)	30 (88.2)
A	1 (2.86)	2 (5.88)	1 (2.94)	2 (5.88)
B	1 (2.86)	1 (2.94)	1 (2.94)	2 (5.88)
F	1 (2.86)	1 (2.94)	0	0

ETV entecavir; *LVD* lamivudine

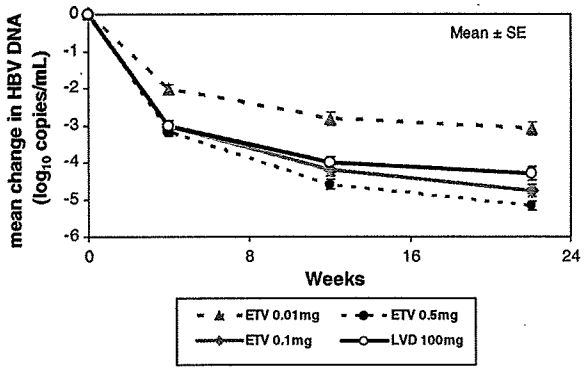

Fig. 1 Mean change from baseline in serum HBV DNA level by PCR assay through 22 weeks in patients treated with entecavir (ETV) 0.01, 0.1, and 0.5 mg and lamivudine 100 mg. Mean change in serum HBV DNA level was plotted as a function of time after the initiation of the protocol therapy (weeks). Data expressed as mean ± SE

significant dose–response relationship between $\log_{10}$ entecavir dose and reduction in $\log_{10}$ serum HBV DNA level ($P < 0.0001$).

Mean change (from baseline) in serum HBV DNA level at week 22 for the lamivudine 100 mg group was −4.29 $\log_{10}$ copies/ml (Fig. 1; Table 2). Estimated mean differences (95% CI) in serum HBV DNA level (after adjustment for baseline level and HBeAg status) were −0.39 (−0.83 to 0.05) $\log_{10}$ copies/ml between the entecavir 0.1 mg and lamivudine 100 mg groups and −0.62 (−1.06 to −0.18) $\log_{10}$ copies/ml between the entecavir 0.5 mg and lamivudine 100 mg groups, indicating the noninferiority of the entecavir 0.1 and 0.5 mg groups to the lamivudine 100 mg group and the superiority of the entecavir 0.5 mg group to the lamivudine 100 mg group ($P = 0.007$) (Table 2). In contrast, the entecavir 0.01 mg group was significantly inferior to the lamivudine 100 mg group (estimated mean difference = 1.20 [0.69–1.71]; $P < 0.0001$) (Table 2).

The secondary efficacy end point of a reduction in serum HBV DNA level 2 $\log_{10}$ copies/ml or more or HBV DNA level less than 400 copies/ml by PCR assay was achieved by 88.6% of patients in the entecavir 0.01 mg group and by 100% of patients in the entecavir 0.1 and 0.5 mg groups at week 22. Ninety-seven percent of patients in the lamivudine 100 mg group achieved this end point at week 22. HBV DNA level less than 0.7 MEq/ml by bDNA assay was achieved by 65.7%, 94.1%, and 100% of patients in the 0.01, 0.1, and 0.5 mg entecavir groups, respectively, and by 93.9% of patients in the lamivudine 100 mg treatment group.

Serologic response

Among HBeAg-positive patients, there was no significant difference between seroconversion rates at week 22 for the entecavir 0.01, 0.1, and 0.5 mg treatment groups (10.0%, 13.3%, and 3.6%, respectively) versus the lamivudine 100 mg treatment group (3.3%; Table 2). All patients who lost HBeAg also experienced HBeAg seroconversion.

Biochemical response

At baseline, elevated serum ALT levels (>1.25 × ULN) were present in more than 90% of patients in all four treatment groups. At week 22, normal serum ALT levels (World Health Organization grade 0, <1.25 × ULN) were recorded in similar proportions of patients in the entecavir 0.01, 0.1, and 0.5 mg treatment groups (75.0%, 85.3%, and 80.0% of patients, respectively) and the lamivudine treatment group (78.1% of patients), with no significant intergroup difference (Table 2).

Response

Response (HBV DNA level <0.7 MEq/ml by bDNA assay, HBeAg loss, and serum ALT level <1.25 × ULN for HBeAg-positive patients and HBV DNA level <0.7 MEq/ml by bDNA assay and serum ALT <1.25 × ULN for HBeAg-negative patients) was achieved by 14.3%, 20.6%, and 15.6% of patients in the entecavir 0.01, 0.1, and 0.5 mg

Table 2 Differences in HBV DNA levels between entecavir dose groups by PCR at week 22 in evaluable subjects

	0.1 mg ETV–0.01 mg ETV ($n = 34$, $n = 35$)	0.5 mg ETV–0.01 mg ETV ($n = 32$, $n = 35$)	0.5 mg ETV–0.1 mg ETV ($n = 32$, $n = 34$)
Estimated difference[a] ($\log_{10}$ copies/ml)	−1.61	−1.95	−0.23
Standard error	0.24	0.24	0.19
95% Confidence interval[b]	−2.20, −1.02	−2.53, −1.37	−0.69, 0.23
P-value	<0.0001	<0.0001	0.227

[a] Estimated differences are regression-adjusted for baseline serum HBV DNA and HBeAg status

[b] 95% Confidence interval is adjusted by modified Bonferroni procedures

ETV entecavir

Table 3 Virology and biochemical responses at week 22 and comparison of entecavir treatment groups with lamivudine in evaluable subjects

Response	ETV 0.01 mg ($n = 35$)	ETV 0.1 mg ($n = 34$)	ETV 0.5 mg ($n = 32$)	LVD 100 mg ($n = 33$)
HBV DNA by PCR assay				
Reduction from baseline at week 22 (log_{10} copies/ml), mean ± S.E.	−3.11 ± 0.18	−4.77 ± 0.17	−5.16 ± 0.13	−4.29 ± 0.18
HBV DNA estimated difference[a] (vs. LVD) (log_{10} copies/ml)	1.20	−0.39	−0.62	–
Standard error	0.26	0.22	0.22	–
95% Confidence interval	0.69, 1.71	−0.83, 0.05	−1.06, -0.18	–
P-value	<0.0001[b]	0.081	0.007[c]	–
HBV DNA by Roche Amplicor™ PCR assay				
Change in log_{10} HBV DNA reduction >2 or HBV DNA <400 copies/ml at week 22, *n* (%)	31 (88.6)	34 (100)	32 (100)	32 (97.0)
P-value (vs. LVD)	0.206	NR[d]	NR[d]	–
HBV DNA by Quantiplex assay				
HBV DNA <0.7 MEq/ml (2.5 pg/ml) at week 22, *n* (%)	23 (65.7)	32 (94.1)	32 (100)	31 (93.9)
P-value (vs. LVD)	0.002	1.000	NR[d]	–
Normalization of ALT levels[e]				
At week 22, *n/n* with abnormal baseline (%)	24/32 (75.0)	29/34 (85.3)	24/30 (80.0)	25/32 (78.1)
P-value (vs. LVD)	0.842	0.439	0.880	–
Loss of HBeAg and seroconversion at week 48[f]				
HBeAg loss, n/n HBeAg positive at baseline (%)	3/30 (10.0)	4/30 (13.3)	1/28 (3.6)	1/30 (3.3)
HBeAg seroconversion	3/30 (10.0)	4/30 (13.3)	1/28 (3.6)	1/30 (3.3)
P-value (vs. LVD)	0.605	0.350	1.000	–
Response[g] at week 22, *n* (%)	5 (14.3)	7 (20.6)	5 (15.6)	3 (9.1)
P-value (vs. LVD)	0.735	0.190	0.480	–

[a] Estimated differences are regression-adjusted for baseline HBV DNA and HBeAg status

[b] Two-sided test indicates inferiority of the entecavir 0.01 mg dose

[c] Two-sided test indicates superiority of the entecavir dose

[d] Not reported because expected counts <5

[e] WHO grade 0, ALT <1.25 × upper limit of normal

[f] Seroconversion was defined as disappearance of HBe-antigen and appearance of HBe-antibody

[g] Response was defined as HBV DNA levels <0.7 MEq/ml, HBeAg negativity and ALT <1.25 × ULN for HBeAg-positive patients and HBV DNA levels <0.7 MEq/ml and ALT <1.25 × ULN for HBeAg-negative patients

ETV entecavir

LVD lamivudine

treatment groups, respectively, and by 9.1% of patients in the lamivudine treatment group at week 22, and there were no significant differences in the rates of response between the four treatment groups (Table 2).

Resistance analysis

During the treatment period, serum HBV DNA level increased by 1 log_{10} copies/ml or more from its nadir in one patient in the entecavir 0.01 mg group and one patient in the lamivudine 100 mg group. Nucleotide sequence analysis of the DNA polymerase coding region, using viral samples collected from these two patients at day 1 and at week 22, revealed no lamivudine-resistance substitutions (rt180 and rt204 amino acid residues) [17, 18] or entecavir-resistance substitutions (rt184, rt202, and rt250 amino acid residues) [19].

Safety

During the study, adverse events were experienced by similar proportions of patients in the entecavir 0.01, 0.1, and 0.5 mg groups and the lamivudine 100 mg treatment group (97.1%, 97.1%, 91.2%, and 100.0%, respectively). Most adverse events were of mild or moderate intensity (grade 1/2) and transient. The most frequently reported adverse events (affecting ≥ 10% of patients in any one treatment group) included nasopharyngitis, headache, and

Table 4 Summary of adverse events and laboratory abnormalities during the 24-week blinded treatment phase

	ETV 0.01 mg ($n = 35$)	ETV 0.1 mg ($n = 34$)	ETV 0.5 mg ($n = 34$)	LVD 100 mg ($n = 34$)
Any adverse events	34 (97)	33 (97)	31 (91)	34 (100)
Most frequent clinical adverse events,[a] *n* (%)				
Nasopharyngitis	9 (25.7)	10 (29.4)	11 (32.4)	10 (29.4)
Headache	6 (17.1)	7 (20.6)	2 (5.9)	7 (20.6)
Diarrhea	1 (2.9)	1 (2.9)	4 (11.8)	4 (11.8)
Grade 3/4 clinical adverse events, *n* (%)	0	0	1 (2.9)	1 (2.9)
Grade 3/4 laboratory adverse events, *n* (%)	2 (5.7)	4 (11.8)	2 (5.9)	4 (11.8)
Any serious adverse events, *n* (%)	0	1 (2.9)	2 (5.9)	1 (2.9)
Discontinuations due to adverse events,[b] *n* (%)	0	0	1 (2.9)	1 (2.9)
ALT flares,[c] *n* (%)	0	1 (2.9)	1 (2.9)	2 (5.9)
Death, *n* (%)	0	0	0	0

[a] Occurring in at least 10% of patients

[b] One patient treated with ETV 0.5 mg discontinued the study drug due to hepatic cirrhosis. One patient treated with lamivudine discontinued due to increased ALT

[c] ALT flare defined ALT >2 × baseline and 10 × ULN

ETV entecavir

LVD lamivudine

diarrhea (Table 4). Grade 3/4 clinical adverse events occurred in one patient in the entecavir 0.5 mg group (colon carcinoma) and one patient in the lamivudine group (anal ulcer); neither of these events was considered to be related to the study drug. Serious adverse events were limited to the above-mentioned case of colon carcinoma, serum ALT elevation (entecavir 0.1 mg group [$n = 1$], entecavir 0.5 mg group [$n = 1$]), and serum aspartate aminotransferase (AST)/ALT elevation (lamivudine 100 mg group [$n = 1$]), but these were not considered to be causally related to the study drug and did not necessitate treatment discontinuation. Transient ALT flares (serum ALT >2 × baseline level and >10 × ULN) occurred in four patients (entecavir 0.1 mg group [$n = 1$], entecavir 0.5 mg group [$n = 1$], and lamivudine 100 mg group [$n = 2$]) and were associated with HBV DNA level decreases of 2 $\log_{10}$ copies/ml or more. None of the ALT flares were associated with hepatic decompensation and serum ALT and AST levels recovered to less than 1.25 × baseline level on continuation of the study treatment.

Discussion

The global ETV-005 study reported that entecavir was superior to lamivudine at reducing viral load in nucleoside-naive patients with CHB infection [15]. We conducted the present study, using an identical design to the ETV-005 study, to determine whether the findings from this earlier study are applicable to Japanese patients. In keeping with the previous findings, our results indicate that entecavir produces a dose-related reduction in serum HBV DNA level ($0.01 < 0.1 \leq 0.5$ mg) in nucleoside-naive Japanese patients with CHB; the log dose–response curves for the reduction in serum HBV DNA level with entecavir in the two studies were similar, with estimated regression curve slopes of –1.24 (Japanese study) and –1.32 (global study). In addition, both studies demonstrated the noninferiority of the entecavir 0.1 mg group compared with the lamivudine 100 mg group and the superiority of the entecavir 0.5 mg group compared with the lamivudine 100 mg group. The demonstration of a dose–response relationship for entecavir and the superiority of the entecavir 0.5 mg dose over lamivudine confirm that the antiviral activity of entecavir in Japanese patients is similar to that observed in study ETV-005. In a previous study, Ono et al. [14] demonstrated that the in vitro potency of entecavir was up to 2,200 times greater than that of lamivudine. The results presented here substantiate these earlier in vitro data and confirm the greater potency of entecavir over lamivudine in patients with CHB.

Serum ALT normalization rates with entecavir 0.5 mg and lamivudine 100 mg (~80%) were higher in the present study than those reported in the ETV-005 study (entecavir 0.5 mg, 69.0%; lamivudine 100 mg, 59.1%) [15]. In keeping with previous findings [20, 21], the incidence of entecavir-associated serum ALT flares in Japanese patients was low. The serum ALT flares occurred against a background of 2 $\log_{10}$ copies/ml or more reductions in serum

HBV DNA level, and serum ALT levels subsequently normalized without discontinuation of entecavir. Therefore, the serum ALT flare noted here may indicate recovery of the host's immune response arising from the reduction in HBV viral titer [22, 23]. ALT flares have been reported after the discontinuation of entecavir therapy [15, 16], thus necessitating long-term follow-up to identify possible posttreatment viral rebound.

In conclusion, the results of this dose-ranging study demonstrate a clear dose–response relationship for entecavir in terms of mean HBV DNA level reduction at week 22. Entecavir 0.5 mg was significantly more effective than lamivudine 100 mg in reducing HBV DNA levels in nucleoside-naive Japanese adult patients with CHB. At this dose level, entecavir treatment resulted in serum HBV DNA levels of less than 400 copies/ml in 100% of patients and normalization of serum ALT levels in 80% of patients after 22 weeks. Moreover, entecavir 0.5 mg once daily was well tolerated and showed a comparable safety profile to lamivudine.

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Evaluation of portosystemic collaterals by MDCT-MPR imaging for management of hemorrhagic esophageal varices

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ABSTRACT

Objective: To study the correlation between changes in portosystemic collaterals, evaluated by multidetector-row computed tomography imaging using multiplanar reconstruction (MDCT-MPR), and prognosis in patients with hemorrhagic esophageal varices (EV) after endoscopic treatment.
Methods: Forty-nine patients with primary hemostasis for variceal bleeding received radical endoscopic treatment: endoscopic injection sclerotherapy (EIS) or endoscopic variceal ligation (EVL). Patients were classified according to the rate of reduction in feeding vessel diameter on MDCT-MPR images, into the narrowing ($n=24$) and no-change ($n=25$) groups. We evaluated changes in portosystemic collaterals by MDCT-MPR before and after treatment, and determined rebleeding and survival rates.
Results: The left gastric and paraesophageal (PEV) veins were recognized as portosystemic collaterals in 100 and 80%, respectively, of patients with EV on MDCT-MPR images. The rebleeding rates at 1, 2, 3, and 5 years after endoscopic treatment were 10, 15, 23, and 23%, respectively, for the narrowing group, and 17, 24, 35, and 67%, respectively, for the no-change group ($P=0.068$). Among no-change group, the rebleeding rate in patients with large PEV was significantly lower than that with small PEV ($P=0.027$). The rebleeding rate in patients with small PEV of the no-change group was significantly higher than that in the narrowing group ($P=0.018$). There was no significant difference in rebleeding rates between the no-change group with a large PEV and narrowing group ($P=0.435$).
Conclusion: Changes in portosystemic collaterals evaluated by MDCT-MPR imaging correlate with rebleeding rate. Evaluation of portosystemic collaterals in this manner would provide useful information for the management of hemorrhagic EV.

1. Introduction

Variceal bleeding is a serious adverse event in patients with liver cirrhosis. Patients surviving the first episode of variceal bleeding have a greater than 60% risk of recurrent hemorrhage within 1 year of the initial episode [1]. All patients surviving a variceal bleed should therefore receive radical treatment to prevent rebleeding. The combination therapy of pharmacological treatment and endoscopic variceal ligation (EVL) is generally considered to prevent variceal rebleeding [2–4]. However, some studies showed that narrowing of feeding vessels by embolization with endoscopic injection sclerotherapy (EIS) reduced the recurrence of esophageal varices (EV) [5–7].

Although some studies suggested a close relationship between changes to feeding vessels and EV recurrence after endoscopic therapy [8], little is known about the portosystemic collaterals and their association with rebleeding of hemorrhagic EV. New endoscopic methods to treat and monitor the treatment effect in these patients are clearly needed. The portal venous system has been evaluated by invasive methods such as angiography and percutaneous transhepatic portography (PTP). However, advances in computed tomography (CT) for diagnostic imaging allow useful information about portosystemic collaterals to be obtained by multidetector-row CT imaging (MDCT). Previous evaluation by MDCT-multiplanar reconstruction (MPR) imaging before and after endoscopic treatment showed a close relationship

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between changes in feeding vessels and prognosis after endoscopic therapy [9].

The present retrospective study was designed to determine the relationship between portosystemic collaterals and prognosis of hemorrhagic EV using MDCT-MPR imaging.

2. Materials and methods

2.1. Patients

Sixty consecutive patients with hemorrhaged EV were admitted to our institution from January 2000 to March 2007. Two patients died of hemorrhagic shock while 58 patients underwent emergency endoscopic examination after reaching a stable condition. Active bleeding was detected in 40 patients and in the remaining 18 patients spontaneous hemostasis was evaluated as a red plug in 4 patients and as a white plug in 14 patients. As a rule, primary hemostasis was induced at the bleeding point by endoscopic variceal ligation (EVL). All 40 patients treated by EVL achieved primary hemostasis. However, 2 refused radical treatment, one underwent liver transplantation after endoscopic hemostasis, and 6 patients died of liver failure. Thus, 49 patients (18 with spontaneous hemostasis and 31 with primary hemostasis) underwent radical treatment (Fig. 1). Radical endoscopic treatment was performed after estimating the general condition of the patient, liver function, renal function, portosystemic collaterals, and hepatocellular carcinoma (HCC) by MDCT-MPR imaging. Endoscopic injection sclerotherapy (EIS), in which the sclerosant (5% ethanolamine oleate) is injected into the varices, was generally used as the radical treatment. However, EVL was selected for patients with progressive HCC, poor liver function, poor renal function, and narrow EVs. Consequently, EIS was performed in 37 patients while EVL in 12 patients. MDCT-MPR imaging was also used to evaluate the effect of endoscopic treatment. None of the 49 patients was on β-blocker medication during the follow-up period. Fig. 1 shows the algorithm for selection of patients with acute variceal bleeding for treatment. Fig. 2 shows examples of variceal bleeding and hemostasis with EVL.

Table 1 lists the clinical characteristics of patients. Endoscopic findings of the EVs were evaluated according to the classifica-

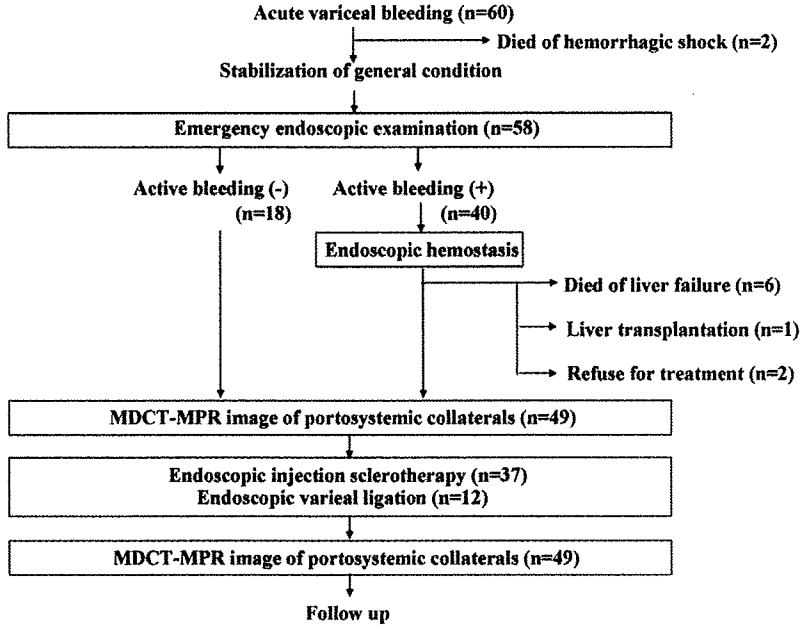

Fig. 1. Algorithm for selection of patients with acute variceal bleeding for treatment.

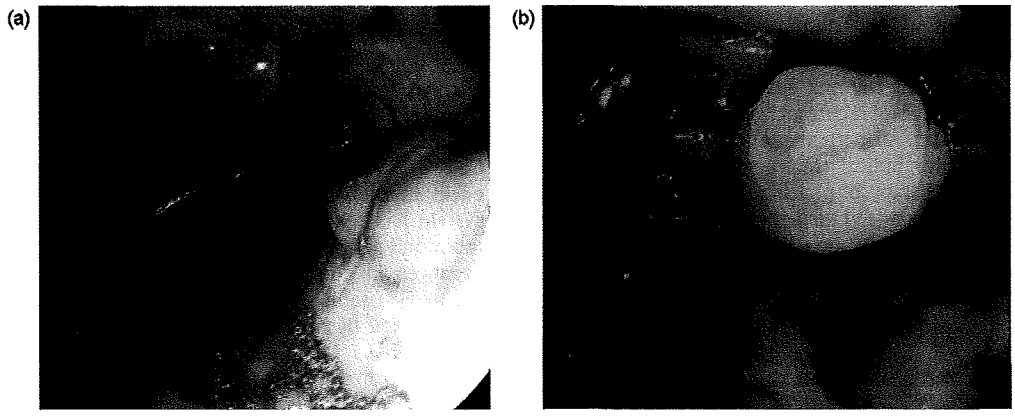

Fig. 2. Endoscopic findings of hemorrhagic esophageal varices. (a) spurting bleeding and (b) endoscopic variceal ligation.

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Table 1
Clinical characteristics of patients ($n = 49$).

Gender (male/female)	38/11
Age (years) (mean ± SD)	58 ± 11 (29–80)
Etiology (HBV/HCV/alcohol/others)	8/30/8/3
Child-Pugh classification (A/B/C)	5/34/10
Variceal size (F1/F2/F3)[a]	10/28/11
Red color sign (RC0/RC1/RC2/RC3)[a]	6/27/15/1
HCC (presence/absence)	21/28
HCC stage (1/2/3/4)[b]	0/4/7/10
Vp (0,1,2/3,4)[b]	32/17
Total bilirubin (≤2/>2) (mg/dl)	34/15
Serum albumin (≥3.0/<3.0) (g/dl)	26/23
Cr (<1.0/≥1.0) (mg/dl)	44/5
Previous treatment for esophageal varices (yes/no)	18/31

HCC: hepatocellular carcinoma.
[a] Evaluated according to the classification system of the Japanese Society for Portal Hypertension and Esophageal Varices.
[b] Classified according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [11].

tion system of the Japanese Society for Portal Hypertension and Esophageal Varices [10]. The form (F) of EV was classified as complete eradication after treatment (F0), small straight (F1), enlarged tortuous (F2), and large coiled-shaped (F3). Red color sign (RC) was defined as endoscopically detected dark red spots on the mucosa of the lower esophagus. To evaluate the risk of hemorrhage and provide a rough estimate of intravascular pressure within the EV, RC was classified into four grades: RC0: no mucosal coloring; RC1: a few localized red spots; RC2: between RC1 and RC3; and RC3: several mucosal red spots throughout the circumference of the lower esophagus. Endoscopic findings of EV before treatment were F1 in 10 patients, F2 in 28 patients, and F3 in 11 patients, as well as RC0 in 6 patients, RC1 in 27 patients, RC2 in 15 patients, and RC3 in 1 patient. HCC was present in 21 patients and absent in 28 patients. HCCs were classified according to the General Rules for the Clinical and Pathological Study of Primary Liver Cancer [11]. The stage of HCC before treatment was evaluated as stage II in 4 patients, stage III in 7 patients, and stage IV in 10 patients; no patients showed stage I HCC. The severity of tumor thrombus in the portal vein was Vp 0, 1, or 2 in 32 patients and Vp 3 or 4 in 17 patients.

The institutional review board approved this study, which was based on the Declaration of Helsinki as declared by the World Health Organization. All subjects gave informed consent.

2.2. EVL procedure

The endoscopic variceal device (Sumitomo Bakelite, Tokyo, Japan) consists of an outer housing cylinder, an inner banding cylinder, and an elastic O-ring. After confirming variceal bleeding and the need for banding, the endoscope was removed and the banding device assembled by securing the housing cylinder to the end of the scope. The target varices were identified and the scope advanced under direct vision until the banding cylinder was in full 360° contact with the varices. Suction was then applied by depressing the endoscopic aspiration control valve, thus drawing the varices and surrounding mucosa into the banding chamber. Once the chamber was completely filled, the elastic band over the varices was ejected. The engorged varices were thus strangulated at the mucosal junction [12].

2.3. EIS procedure

The EIS technique was designed to embolize EV feeding vessels within the portosystemic collaterals by injecting a sclerosing agent. A balloon, referred to as the oral side balloon in this study, was attached to the tip of an endoscope (model GIF-XQ 240, Olympus, Tokyo) and inflated as iopamidol contrast medium (Iopamiron; Schering, Berlin, Germany) was injected to prevent the sclerosant agent (5% ethanolamine oleate) from flowing out of the varices into the systemic circulation. The flow of sclerosant was monitored using X-ray fluoroscopy from the start of injection into the EV. The injection was terminated when the sclerosant started to fill the portosystemic collaterals.

2.4. CT examination

When the CT located the liver, the contrast medium: 100 ml of iopamidol 300 heated to 37 °C, was injected using a power injector (Auto Enhance A-250; Nemoto-Kyorindo, Tokyo), at a rate of 4.0 ml/s through a 22-gauge IV catheter inserted into an antecubital vein. Four sets of images were acquired in a craniocaudal direction at 20, 40, 65, and 180 s after injection of the contrast medium. The first and second acquisitions were used for hepatic arterial phase imaging, the third acquisition for portal venous phase imaging, and the fourth acquisition to image the hepatic venous phase. The third set of images was obtained during 20-s breath holding, while those of other acquisitions were achieved during 10-s breath holding. This protocol is routinely used in all patients with chronic liver disease at our institution, and the data of the third acquisition are used for construction of three-dimensional (3D) images of the portosystemic collaterals. All scans were performed on a LightSpeed QX/I CT scanner (Generic Electric Medical Systems, Milwaukee, WI) using the high-quality scan mode, at 1.25-mm slice thickness, and reconstruction intervals of 0.625 mm for portal venous phase imaging. MDCT was performed using a Virtual Place Advance (AZE, Tokyo) [13]. There are currently three reformatting techniques available, but MPR was used for image reconstruction in this study. CT examination was performed in every patient, within 1 month prior to endoscopic treatment and after the final session.

2.5. Evaluation of portosystemic collaterals

Portosystemic collaterals were assessed independently on MDCT-MPR imaging before and after endoscopic treatment for EV. The diameter of the main portosystemic collateral vessel such as the left gastric vein (LGV), paraesophageal vein (PEV), which drains the EV, was measured at the thickest portion of the vessel before and after endoscopic treatment. Changes in the feeding vessels after endoscopic treatment were estimated by calculating the rate of reduction, using the following formula: rate of reduction of the diameter of feeding vessel (%) = [(diameter of feeding vessel before endoscopic treatment − diameter of feeding vessel after endoscopic treatment)/diameter of feeding vessel before endoscopic treatment] × 100. Patients were divided into two groups according to the rate of reduction of the vessel diameter. Patients with a reduction rate of >20% were classified as the narrowing group, while those with a rate of ≤20% were classified into the no-change group. The cutoff rate of 20% represented the median reduction of the feeding vessel diameter in this series. Patients who showed no enhancement of the feeding vessel on MDCT were included in the narrowing group. Moreover, based on the diameter of PEV, which drains the EV, patients were divided into two groups: the large PEV group (≥3 mm) and the small PEV group (<3 mm). A cutoff diameter of 3 mm represented the median PEV diameter. Patients with PEV too narrow to be recognized on MDCT were classified as the small PEV group.

2.6. Follow-up

Radical endoscopic treatment such as EIS and EVL was repeated after one to two weeks, when needed to complete one treatment series and eliminate all varices. Additional treatment was applied until EV was classified as F0 endoscopically. Rebleeding after endo-

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scopic treatment was also assessed by endoscopy performed at 6-month intervals after treatment. We defined rebleeding of EV as the primary endpoint and survival as the secondary endpoint. The relationship between hemodynamic changes in the portosystemic collaterals and prognosis of patients with hemorrhagic EV after endoscopic treatment was analyzed by MDCT-MPR imaging and image analysis.

2.7. Statistical analysis

The cumulative rebleeding rate and cumulative survival rate between the groups classified according to rate of reduction after treatment were determined using the Kaplan–Meier method and statistical software (SPSS, Chicago, IL). Significance was tested using a generalized log-rank test and *t*-test. A *P* value < 0.05 was regarded as statistically significant.

3. Results

3.1. Portosystemic collaterals recognized by MDCT

Portosystemic collaterals were recognized on MDCT-MPR images of all patients with EV (Table 2). The LGV was the main EV feeding vessel in all patients, with a mean diameter of 5.8 mm. PEV was detected by MDCT-MPR in 80% of patients, with a mean diameter of 3.4 mm.

Twenty-four patients were classified as the narrowing group while 25 patients were considered the no-change group based on the rate of diameter change of portosystemic collaterals after endoscopic treatment. Fig. 3a and b shows typical MPR images of portosystemic collaterals in a patient of the narrowing group and another of the no-change group. Of patients who underwent EIS, 24 of the narrowing group and 13 of the no-change group, while all patients who underwent EVL were of the no-change group. However, the method of endoscopic treatment did not influence the rebleeding rate.

3.2. Cumulative rebleeding rates

The cumulative rebleeding rates of EV after endoscopic treatment were 14, 20, 28, and 42% at 1, 2, 3, and 5 years after treatment, respectively, for all patients (Fig. 4a). The median follow-up period was 26 months. Fig. 4b shows the cumulative rebleeding rates according to changes in the portosystemic collaterals after endoscopic treatment. The rates at 1, 2, 3, and 5 years after endoscopic treatment were 10, 15, 23, and 23%, respectively, for patients in the narrowing group, and 17, 24, 35, and 67%, respectively, for no-change group patients. There were no significant differences in cumulative rebleeding rates between the narrowing and no-change groups ($P = 0.068$).

3.3. Cumulative rebleeding rates with or without PEV

Patients in both groups were divided further based on PEV diameter, using a cutoff value of 3 mm. There were no significant differences in cumulative rebleeding rates between the large PEV group and small PEV group after esophageal treatment in patients of the narrowing group. However, in the no-change group, cumulative rebleeding rates at 1, 2, 3, and 5 years after endoscopic treatment were 7, 7, 20, and 60%, respectively, for the large PEV group and 32, 66, 100, and 100%, respectively, for the small PEV group (Fig. 4c). Thus, the diameter of PEV significantly influenced the cumulative rebleeding rates only in the no-change group after endoscopic treatment ($P = 0.027$). The cumulative rebleeding rates of the small PEV group in no-change patients were significantly higher than those measured in the narrowing group ($P = 0.018$). However, no significant change in cumulative rebleeding rates was apparent between the large PEV group in no-change patients and the narrowing group ($P = 0.435$).

Table 2
Portosystemic collaterals identified on multidetector-row computed tomography images.

Portosystemic collaterals	Percentage	Diameter (mm)[a]
Left gastric vein	100% ($n = 49$)	5.8 ± 2.2 (3.3–15)
Paraesophageal vein	80% ($n = 39$)	3.4 ± 1.5 (2.0–8.8)

[a] Data are mean ± SD.

3.4. Cumulative survival rates

The overall cumulative survival rates after endoscopic treatment were 67, 58, 46, and 34% at 1, 2, 3, and 5 years, respectively (Fig. 5). There were no significant differences in cumulative survival rate between the narrowing group and no-change group. The cause of death in this study was HCC in 14 patients, liver failure in 11 patients, other disease in one patient, and variceal rebleeding in 2 patients. The remaining 21 patients are still alive.

4. Discussion

This study retrospectively examined changes in portosystemic collaterals on MDCT-MPR imaging, as well as rebleeding rates and survival rates, after endoscopic treatment of 49 patients with hemorrhaged EV. The cumulative rebleeding rates in patients showing a feeding vessel diameter reduction rate of >20% (narrowing group) after endoscopic therapy tended to be lower than those in no-change group. Moreover, the PEV diameter was significant to the cumulative rebleeding rates only in the no-change group, with rates significantly lower in patients with larger PEV compared to those with smaller PEV. Despite this, there was also no difference in the cumulative rebleeding rates between narrowing group and no-change group with large PEV diameters. These results therefore suggest a close relationship between cumulative rebleeding rates after endoscopic therapy and portosystemic collaterals on MDCT-MPR imaging. No such relationship was identified between cumulative survival rates after endoscopic therapy and portosystemic collaterals.

MDCT achieves more rapid acquisition and higher longitudinal resolution than single-detector CT [14]. The accompanying use of MPR significantly improves the imaging of portosystemic collaterals and the sites of confluence [15,16]. Noninvasive MDCT with MPR before endoscopic therapy for patients with EV thus provides detailed information on the hemodynamics of the portosystemic collaterals [15]. The present study used this technique in patients with bleeding EV to evaluate the relationship between changes in portosystemic collaterals and prognosis [8].

The results of our study using MDCT-MPR imaging in patients with EV emphasized the importance of eradication of feeding vessels by endoscopic treatment in reducing the rebleeding rate. EV rebleeding rates were definitely higher in patients with inadequate eradication of the feeding vessel than in those with adequate eradication. However, even in patients of the former group, such as the no-change group in the present study, EV rebleeding rates were significantly lower in patients with a large PEV than in patients with a small PEV. It is probable that a large-diameter PEV enhances vein drainage from LGV to PEV, thus making EV rebleeding less likely even in patients without complete eradication of the feeding vessel. Thus, obliterating EV on the esophageal mucosa alone by EVL, without eradicating the feeding vessel, might favor low EV rebleeding rates [9].

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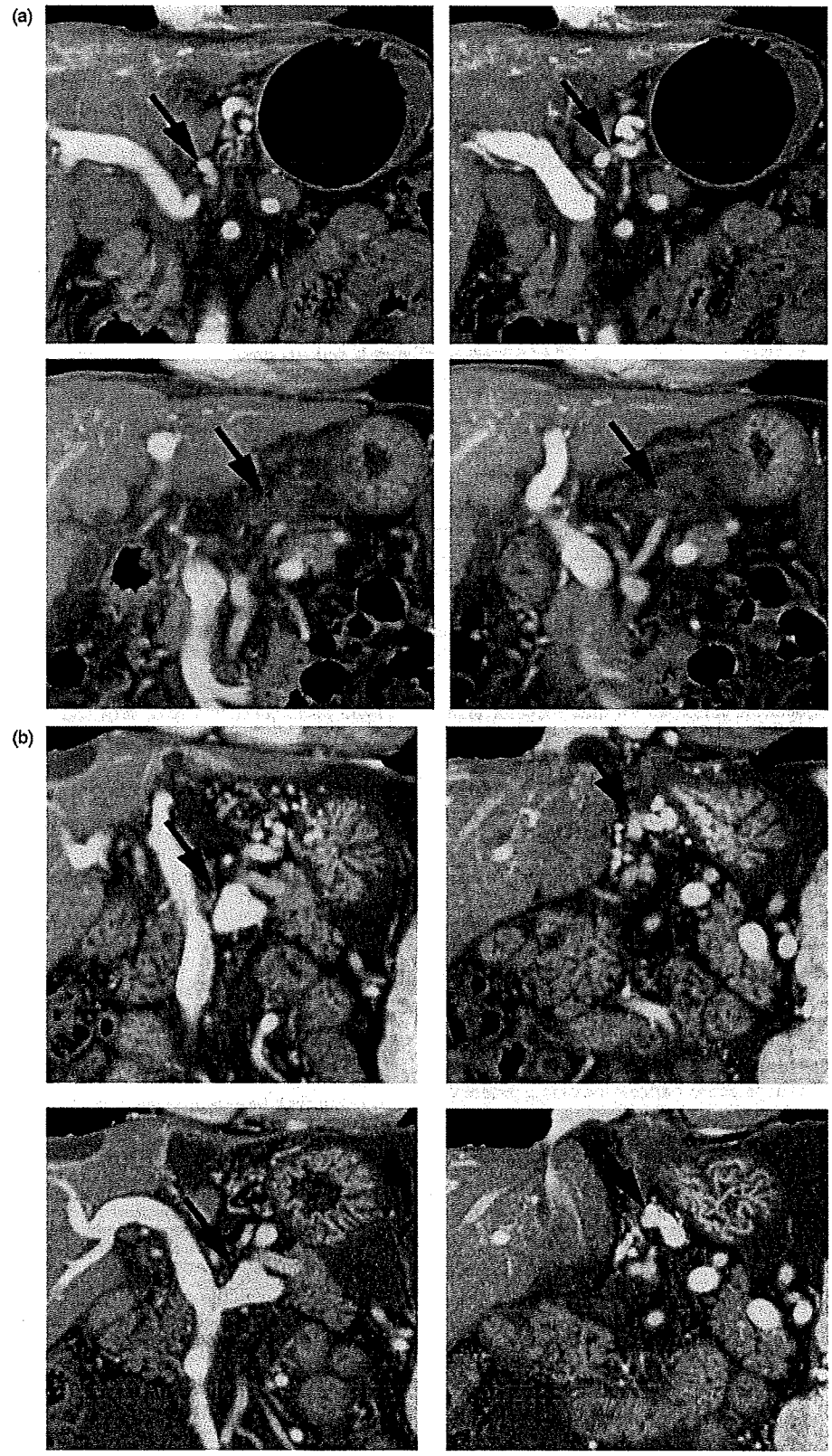

Fig. 3. Typical multiplanar reconstruction images of portosystemic collaterals in representative patients of the narrowing group (a) and no-change group (b), showing left gastric vein (arrow). *Top*: before endoscopic treatment and *Bottom*: after endoscopic treatment. See text for definition of each group.

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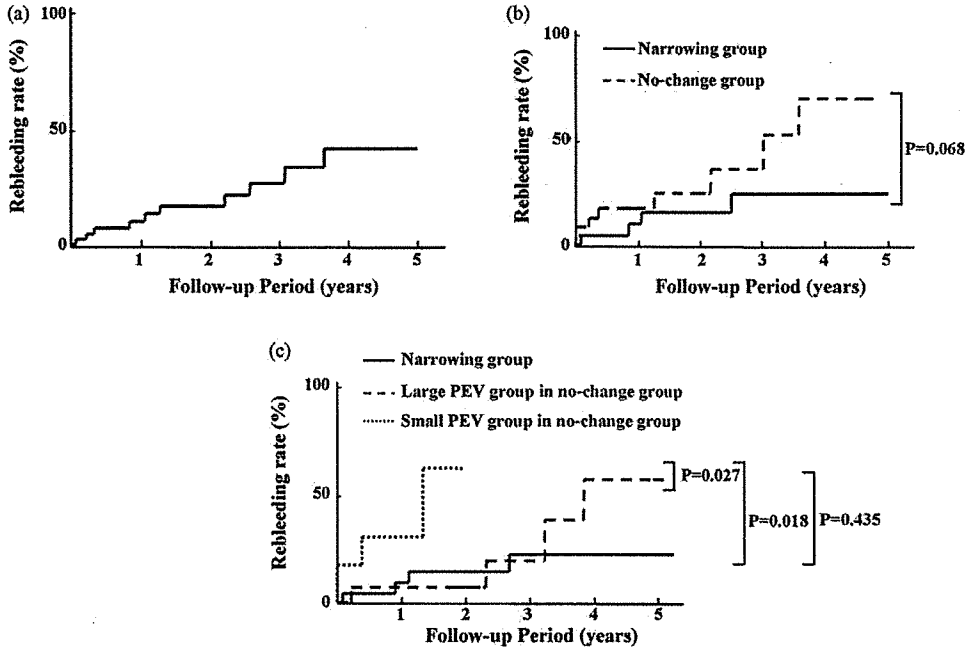

Fig. 4. (a) Cumulative rebleeding rates of hemorrhagic esophageal varices after endoscopic treatment for all patients. (b) Cumulative rebleeding rates of hemorrhagic esophageal varices according to the rate of reduction of feeding vessel after endoscopic treatment. (c) Cumulative rebleeding rates of hemorrhagic esophageal varices according to the rate of reduction of feeding vessel and development of paraesophageal vein after endoscopic treatment.

The guidelines in western countries for treatment of acute variceal bleeding recommend EVL and pharmacological treatment [2–4], as well as reduction in the hepatic venous pressure gradient (HVPG) [17], to prevent rebleeding of EV. On the other hand, our results showed a close relationship between the outcome of endoscopic treatment for variceal bleeding and variability in the portosystemic collaterals. Monitoring changes in these vessels by MDCT-MPR imaging after endoscopic treatment might therefore prove useful to predict outcome and to select the subsequent EV treatment modality, such as EIS or EVL. For example, EIS might be preferable after hemorrhagic EV for patients with a small-diameter PEV to obliterate the feeding vessel completely. On the other hand, EVL only might be necessary for patients with a large-diameter PEV to eradicate EV on esophageal mucosa alone. The results highlight the potential clinical importance of evaluating portosystemic collaterals by MDCT-MPR imaging, used in conjunction with endoscopic treatment, to manage acute variceal bleeding. Although MDCT-MPR images are of high quality, this technology has limitations. The technique can indeed measure vessel diameter, but not the equally important factors of blood pressure and direction of blood flow. Further evaluation of both HVPG and the portosystemic collaterals might therefore be beneficial in assessing the treatment effect.

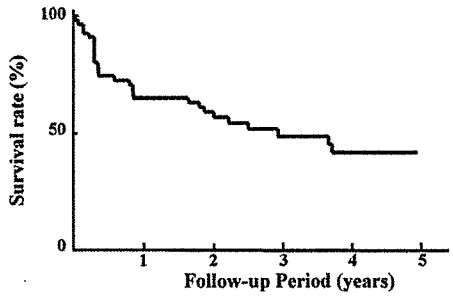

Fig. 5. Cumulative survival rates of hemorrhagic esophageal varices after endoscopic treatment for all patients.

5. Conclusion

Assessing the portosystemic collaterals using MDCT-MPR imaging could help predict EV rebleeding after endoscopic treatment and might prove valuable in the selection of subsequent endoscopic therapy.

Acknowledgments

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study

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Abstract
Background Chronic hepatitis C (CHC) genotype 1b patients with high viral load are resistant to peginterferon (PEG-IFN) and ribavirin (RBV) combination therapy, especially older and female patients.
Methods To elucidate the factors affecting early and sustained viral responses (EVR and SVR), 409 genotype 1b patients CHC with high viral loads who had received 48 weeks of PEG-IFN/RBV therapy were enrolled. The amino acid (aa) sequences of the HCV core at positions 70 and 91 and of the interferon sensitivity determining region (ISDR) were analyzed. Host factors, viral factors, and treatment-related factors were subjected to multivariate analysis.
Results Male gender, low HCV RNA load, high platelet count, two or more aa mutations of ISDR, and wild type of core aa 70 were independent predictive factors for SVR. In patients with over 80% adherences to both PEG-IFN and RBV, male gender, mild fibrosis stage, and wild type of core aa 70 were independent predictors for SVR.
Conclusions Independent predictive factors for SVR were: no aa substitution at core aa 70, two or more aa mutations in the ISDR, low viral load, high values of platelet count, mild liver fibrosis and male gender.

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Keywords Chronic hepatitis C · Peginterferon and ribavirin · Core amino acid · Interferon sensitivity determining region

Abbreviations

CHC	Chronic hepatitis C
PEG-IFN	Peginterferon
RBV	Ribavirin
RVR	Rapid viral response
cEVR	Complete early viral response
LVR	Late viral response
ETR	End of treatment response
NR	Non response
SVR	Sustained viral response
ISDR	Interferon sensitivity determining region
Aa	Amino acid
ALT	Alanine aminotransferase
PLT	Platelet
HCC	Hepatocellular carcinoma

Introduction

A combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy for 48 weeks achieves a sustained viral response (SVR) rate of 40–50% in chronic hepatitis C (CHC) patients with a high viral load of genotype 1 [1–4]. The dose-reduction rate and the frequency of discontinuation of this treatment are high in aged patients [5]. The SVR rate of the therapy is lower in females than males, especially in older patients in Japan [6].

Around 30% of HCV carriers have serum alanine aminotransferase (ALT) levels within the upper limit of normal ranges [7, 8] and HCV carriers with persistently normal serum ALT (PNALT) and serum platelet (PLT) counts of over $15 \times 10^4/mm^3$ show low grade hepatic fibrosis and good prognosis [9]. Before treating HCV carriers, it is very important to predict non-response to PEG-IFN plus RBV therapy because of its medical cost, adverse effects, and its impact on the long term quality of life.

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There are many factors affecting response to IFN monotherapy and PEG-IFN/RBV therapy, including body mass index (BMI) [10, 11], steatosis [12, 13], insulin resistance [14], stage of liver fibrosis [15, 16], total cholesterol (T. Chol), triglyceride (TG), adherence to both PEG-IFN and RBV [17], race [18, 19], age [1, 2, 20], and viral factors including serum quantity of HCV RNA, HCV genotype and substitution of amino acids (aa) in the interferon sensitivity determining region (ISDR, 2209–2248) of the nonstructural protein 5A (NS5A) [21] and in the core protein [22, 23]. Early viral response is an important predictive factor in PEG-IFN/RBV therapy for CHC patients with genotype 1 and high viral loads [24–27].

The aim of this study was to elucidate the valuable predictive factors of SVR in Japanese patients with HCV genotype 1b high viral loads following 48 weeks of PEG-IFN/RBV therapy, focusing on the relationship between aa substitutions in the ISDR and at core aa 70 and 91 and early viral kinetics.

Patients and methods

Selection of patients

This retrospective study was conducted at 15 clinical sites in Japan which are part of the Study Group of Optimal Treatment of Viral Hepatitis supported by the Ministry of Health, Labor and Welfare, Japan. Eligible subjects were CHC patients, who (1) had received liver biopsy; (2) were genotype 1b with high viral load ($\geq$100 KIU/ml by Cobas Amplicor Hepatitis C Virus Test, version 2.0) at the start of PEG-IFN/RBV therapy; (3) received weekly injections of PEG-IFN-α-2b (PEG-INTRON; Shering-Plough, Kenilworth, NJ) of 1.5 μg/kg bw and oral administration of RBV (Rebetol; Shering-Plough) for 48 weeks. The amount of RBV was adjusted based on the subject's body weight; (600 mg for $\leq$60 kg bw, 800 mg for 60–80 kg bw, 1,000 mg for $>$ 80 kg bw); (4) were examined serially for quantitative and qualitative HCV RNA; and (5) the aa sequences at positions 70 and 91 in the core region and of the ISDR in the NS5A had been determined in pretreatment sera.

Hepatitis B virus (HBV) infection, human immunodeficiency virus (HIV) infection, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease were excluded. Histopathological diagnosis was based on the scoring system of Desmet et al. [28]. The definition of alcohol abuse included patients having a history of more than 100 kg of total ethanol intake. Complete blood counts, liver function tests, serum lipids, serum ferritin, serum fibrosis markers, fasting plasma glucose (FPG), and immune reactive insulin (IRI) were examined in most cases. Written informed consent was obtained from all

patients before treatment, and the protocol was approved by the ethics committees in each site.

Study design

Four hundred and nine patients who completed 48 weeks of treatment and were followed for more than 24 weeks after treatment were enrolled in the first study (*Study design 1*).

To elucidate the effect of aa substitution of HCV core and in the ISDR on HCV dynamics, including a rapid viral response (RVR), complete early viral response (cEVR), a late viral response (LVR) and SVR, according to gender and age (<60 years $\geq$ 60 years), 201 of the 409 patients maintaining over 80% adherences to both PEG-IFN and RBV were enrolled in the second study (*Study design 2*).

Nucleotide sequencing of the core and NA5A gene

The nucleotide sequences encoding aa 1–191 (HCV core) and aa 2209–2248 (ISDR) were analyzed by direct sequencing as described by Akuta et al. [22, 27] and Enomoto et al. [21]. In brief, RNA was extracted from the sera and converted to cDNA and two nested rounds of polymerase chain reaction (PCR) were performed. Primers used in the PCR were as follows; (a) Nucleotide sequences of the core region: the first-round PCR was performed with CC11 (sense) and e14 (antisense) primers [22, 27], and the second-round PCR with CC9 (sense) and e14 (antisense) primers [22, 27]. (b) Nucleotide sequences of the ISDR in NS5A: the first-round PCR was performed with ISDR1 (sense) and ISDR2 (antisense) primers [21], and the second-round PCR with ISDR3 (sense) and ISDR4 (antisense) primers [21]. These sequences were compared with the consensus sequence of genotype 1b (HCV-J) [29]. Wild types virus encoded arginine and leucine at aa 70 and 91, respectively, and the aa substitutions were glutamine or histidine at aa 70 and methionine at aa 91.

Viral kinetic study

Serum HCV RNA levels were measured by PCR (Amplicor HCV RNA kit, version 2.0, Roche Diagnostics) using samples taken before treatment and at 4, 12, 24, and 48 weeks after the therapy. SVR was defined as HCV RNA negativity by qualitative analysis by PCR at 24 weeks after the treatment. RVR was defined as HCV RNA negativity at 4 weeks, cEVR as HCV RNA negativity at 12 weeks, LVR as HCV RNA negativity during 13–24 weeks and an end of treatment response (ETR) as HCV RNA negativity at the end of treatment. Patients who remained positive for HCV RNA at the end of the treatment and at 24 weeks after the therapy were defined as non-responders (NR).

Adherences to PEG-IFN and RBV

Adherences to PEG-IFN and RBV were assessed by separately calculating the actual doses of PEG-IFN and RBV received as percentages of the intended dosages. Adherences to PEG-IFN and RBV were divided into two groups; 80%$\leq$ and <80%.

Statistical analysis

All data analyses were conducted using the SAS version 9.1.3 statistical analysis packages (SAS Institute, Cary, NC, USA). Individual characteristics between groups were evaluated by Mann–Whitney *U* test for numerical variables or Fisher's exact test for categorical variables. Variables exhibiting values of $p < 0.1$ in the univariate analysis were subjected to stepwise multivariate logistic regression analysis. The grade of steatosis and iron deposition in liver tissue, BMI, albumin (Alb), low density lipoprotein-cholesterol (LDL-C), homeostasis model assesment-insulin resistance (HOMA-IR), ferritin, and hyaluronic acid were excluded from multivariate logistic regression analysis because of the absence of those data in more than 10% of the patients. All p values of $p < 0.05$ by the two-tailed test were considered statistically significant.

Results

Study design 1

Baseline backgrounds, characteristics and adherences of peginterferon and ribavirin in males and females

The treatment outcome of PEG-IFN and RBV combination therapy depends on gender in Japanese patients, so in addition to aa substitutions in the ISDR in NS5A [21] or at HCV core 70 and 91 [22, 27], we compared the baseline characteristics according to gender (Table 1). Males were younger and the grade of hepatic inflammation was milder in males. The serum levels of LDL-C, PLT count, and aa substitutions of ISDR and at core 70 and 91 did not differ significantly different between males and females. The frequency of no alcohol abuse was significantly ($p < 0.0001$) higher in females than males (Some of them are not described in Table 1).

The rates of over 80% adherences to PEG-IFN and RBV were significantly lower ($p = 0.0066$, $p < 0.00001$, respectively) in females than males. Only in those above 60 years did the rate of over 80% adherence to PEG-IFN not differ significantly between males and females, but the rate of over 80% adherence to RBV was significantly lower ($p = 0.035$) in females than males (Table 1).

Table 1 Backgrounds and characteristics of male and female patients

Factors	Gender		*p* value
	Male	Female	
No. of patients	256 (62.6%)	153 (37.4%)	
Age			
Median (range)	53 (18–73)	59 (23–75)	0.00001
F stage			
F0–2	206 (80.5%)	119 (77.8%)	0.592
F3–4	50 (19.5%)	34 (22.2%)	
Grade (A factor)			
A0–1	163 (63. 7%)	79 (51.6%)	0.026
A2–3	93 (36.3%)	74 (48.4%)	
HCV RNA load 0 week (KIU/mL)			
Median (range)	1500 (100–5000 <)	1280 (100–5000<)	0.384
ALT 0 week (IU/L)			
Median (range)	74.5 (16–504)	59 (19–391)	0.001
BMI			
Median (range)	23.6 (17.5–31.2)	22.1 (16.1–33.9)	0.00033
Alb (g/dL)			
Median (range)	4.0 (3.0–5.2)	3.8 (3.0–4.8)	0.011
LDL-C (mg/dL)			
Median (range)	97 (30–185)	90 (34–174)	0.612
T-Chol (mg/dL)			
Median (range)	167 (85–273)	176 (114–261)	0.0016
PLT count ($\times 10^4/mm^3$)			
Median (range)	17.0 (8.0–31.9)	16.4 (8.1–39.9)	0.350
Amino acid mutation of ISDR			
0–1	200 (78.1%)	121 (79.1%)	0.608
2≤	56 (21.9%)	32 (20.9%)	
Amino acid substitution of core 70			
Wild	177 (69.1%)	114 (74.5%)	0.261
Mutant	79 (30.9%)	39 (25.5%)	
Amino acid substitution of core 91			
Wild	153 (59.8%)	98 (64.1%)	0.403
Mutant	103 (40.2%)	55 (35.9%)	
PEG-IFN adherence			
<80%	41 (17.7%)	42 (30.4%)	0.0066
80%≤	190 (82.3%)	96 (69.6%)	
Ribavirin adherence			
<80%	54 (23.6%)	73 (52.1%)	<0.00001
80%≤	175 (76.4%)	67 (47.9%)	
Age: <60 years			
PEG adherence			
<80%	30 (17.8%)	23 (31.5%)	0.027
80%≤	139 (82.2%)	50 (68.5%)	
Ribavirin adherence			
<80%	27 (16.2%)	31 (42.5%)	0.000029
80%≤	140 (83.8%)	42 (57.5%)	
Age: 60 years≤			
PEG adherence			
<80%	11 (17.7%)	19 (29.2%)	0.147
80%≤	51 (82.3%)	46 (70.8%)	
Ribavirin adherence			
<80%	27 (43.5%)	42 (62.7%)	0.035
80%≤	35 (56.5%)	25 (37.3%)	

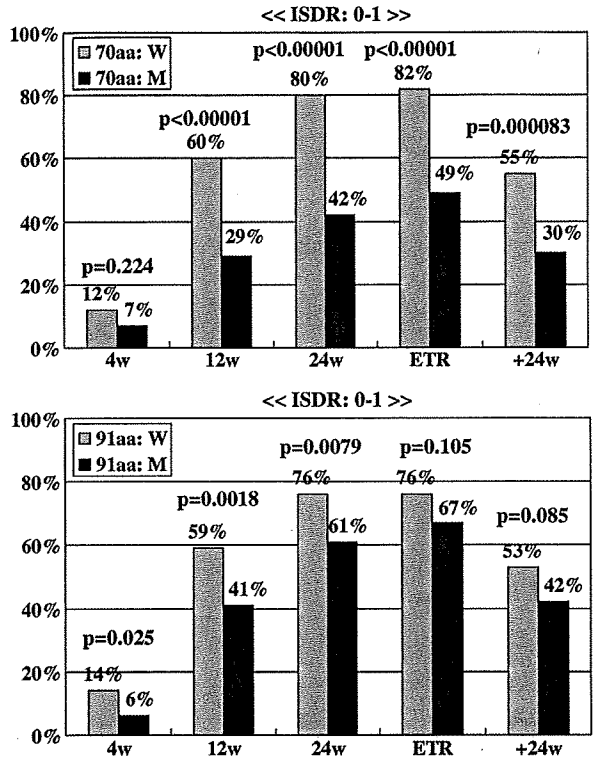

Fig. 1 Relationship between time course of serum HCV RNA negativity and amino acid substitutions in the ISDR and core amino acids 70 and 91. For cases with no or only one amino acid (aa) change in the ISDR, the rates of cEVR, LVR, ETR and SVR were significantly higher in patients with wild type core aa 70 but only the rates of RVR, cEVR, and LVR were significantly higher in patients with wild type core aa 91

Amino acid substitutions

There were no significant differences in the frequency of aa substitutions in the ISDR between males and females. Core aa substitutions at positions 70 and 91 were as follows; 291 (71.1%) were wild type and 118 (28.9%) were mutant at core aa 70, and 251 (61.4%) were wild type and 158 (38.6%) were mutant at core aa 91. There were no significant differences between males and females and between patients below and above 60 years of age.

Virological responses and aa substitutions

The rate of RVR did not differ significantly between males and females. However, more male patients showed HCV RNA negativity at 12 weeks (males vs. females; 60.7 vs. 48.4%, $p = 0.018$), 24 weeks (76.8 vs. 64.2%, $p = 0.0078$) and 48 weeks (78.2 vs. 68.6%, $p = 0.049$), and the proportion of male patients in SVR was significantly higher than that of females (61.3 vs. 37.3%, $p < 0.00001$).

RVR, cEVR and SVR rates were significantly higher in patients with two or more aa mutations in the ISDR compared to patients having no or one aa substitution in that region (20 vs. 11%, $p = 0.044$; 71 vs. 52%, $p = 0.0021$; 66 vs. 49%, $p = 0.0054$, respectively). AA substitution at core position 70 resulted in significantly lower rate of cEVR, LVR, ETR, and SVR (40 vs. 63%, $p = 0.000037$; 51 vs. 81%, $p < 0.00001$; 56 vs. 83%, 41 vs. 57%; $p < 0.00001$, $p = 0.0031$, respectively). Although the patients with the wild type aa at core 91 showed significantly higher rates of RVR and cEVR, the rate of SVR was not significantly higher in those patients ($p = 0.054$).

SVR rates were 30% for patients with no or one aa substitution in the ISDR and the core aa 70 substitution, and were significantly lower compared to those with the wild type aa core 70 (Fig. 1). These findings were not confirmed in patients with no or one aa substitution in the ISDR and the core aa 91 substitution (Fig. 1).

Factors affecting SVR by univariate analysis

Univariate analysis identified nine parameters that influenced non-SVR significantly: female gender, older age, advanced staged liver fibrosis, high viral load, low serum Alb level, low PLT count, no or one aa substitution in the ISDR, aa substitution at core aa 70, and low adherence to RBV (Table 2). The frequency of steatosis and HOMA-IR were significantly ($p = 0.0057$, $p < 0.00001$, respectively) lower in patients with SVR compared with non-SVR (data not shown). However, these factors were not entered in the multivariate analysis because of the absence of the data in many cases.

Factors affecting RVR, cEVR, and SVR by multivariate logistic regression analysis

Multivariate analysis identified four parameters that influenced RVR independently: low HCV RNA load, low serum ALT level, two or more aa mutations in the ISDR and the wild type aa at core position 91 (Table 3).

Concerning cEVR, male gender, mild fibrosis stage, low HCV RNA load, two or more aa mutations in the ISDR, and the wild type aa at core positions 70 and 91 were independent predictors (Table 3).

Concerning SVR, male gender ($p < 0.0001$), low HCV RNA load ($p = 0.013$), high PLT count ($p = 0.0019$), two or more aa mutations in the ISDR ($p = 0.024$), and wild type core aa 70 ($p = 0.0045$) were found to be independent predictors (Table 3).

The predictive values of the combination of gender, PLT count, ISDR and core aa 70 are shown in Fig. 2a. In male patients having PLT of $<15 \times 10^4/mm^3$, and, no or one aa substitution in the ISDR, the SVR rate was 68% when core 70