Table 1 *Continued*

Clinical feature	Before interferon	At detection of HCC	*P*-value
Serological group before interferon [*n* (%)]			
Group 1	6 (33%)	—	—
Group 2	12 (67%)	—	—
Hepatic function [median (range)]			
Platelet ($\times 10^4/mm^3$)	11.6 (6.6–31.0)	16.5 (7.3–31.0)	<0.0001
Total bilirubin (mg/dL)	0.7 (0.3–1.5)	0.7 (0.3–16.8)	0.32
Albumin (g/dL)	4.2 (3.3–5.0)	4.4 (3.2–5.2)	0.10
Aspartate aminotransferase (IU/L)	78 (29–288)	29 (14–159)	<0.0001
Alanine aminotransferase (IU/L)	109 (24–295)	23 (8–178)	<0.0001
Prothrombin time	81 (49–124)	89 (68–137)	0.03
Indocyanine green R_{15} (%)	15.0 (5.0–45.0)	10.6 (3.1–27.4)	0.0009
Histologic fibrosis staging [*n* (%)]			
F0	0 (0%)	1 (6%)	
F1	9 (26%)	3 (19%)	
F2	10 (29%)	8 (50%)	
F3	10 (29%)	2 (13%)	
F4	6 (17%)	2 (13%)	0.11
Histologic activity grade [*n* (%)]			
A0	0 (0%)	6 (38%)	
A1	7 (23%)	8 (50%)	
A2	17 (57%)	2 (13%)	
A3	6 (20%)	0 (0%)	0.001
Treatment-related variables			
Treatment periods (weeks) [median (range)]	24 (2–31)	—	—
Interferon type [*n* (%)]			
α	36 (95%)	—	—
β	2 (5%)	—	—
Total amount of interferon [median (range)]	480 (126–846)	—	—
Prior interferon therapy [*n* (%)]			
Positive	2 (5%)	—	—
Tumor-related variables			
Number of tumors [*n* (%)]			
Solitary	—	31 (82%)	—
Multiple (range)	—	7 (18%)	—
Maximum tumor size (mm)			
Median		30 (12–150)	
≤30 [*n* (%)]	—	21 (57%)	—
>30 [*n* (%)]	—	16 (43%)	—
Alpha-fetoprotein (ng/mL) [*n* (%)]			
>20	4 (16%)	15 (41%)	0.07
PIVKA-II (AU/mL) [*n* (%)]			
>0.063	0 (0%)	13 (43%)	0.01
Differentiation of HCC [*n* (%)]			
Well-differentiated	—	11 (44%)	—
Moderately differentiated	—	11 (44%)	—
Poorly differentiated	—	2 (8%)	—
Combined type	—	1 (4%)	
Period until development of HCC (years) [median (range)]	—	4.7 (1.4–9.0)	—
Period of medical follow-up (months) [median (range)]	—	3 (0.5–57)	—
First treatment for HCC‡ [*n* (%)]			
Resection	—	16 (43%)	—
Local ablation	—	10 (27%)	—
Transarterial treatment	—	11 (30%)	—

PIVKA-II, protein induced by vitamin K absence or antagonist-II; R_{15}, indocyanine green retention rate at 15 min.
†Ethanol intake ≥80 g/day for ≥5 years. ‡One patient has not yet undergone treatment for HCC.

during which checks for HCC were performed using tumor markers and/or imaging modalities.

Differences between data obtained before IFN therapy and at detection of HCC were evaluated using the Wilcoxon signed-rank test. All *P*-values presented in this report are of the two-tailed type. Differences at $P < 0.05$ were considered statistically significant. All analyses were conducted using SPSS 8.0 J (SPSS Inc. Chicago, IL, USA).

RESULTS

In the first data collection, a total of 7715 patients with chronic hepatitis C were identified who had been treated with IFN and followed up for more than 1 year after the termination of IFN therapy from July 1988 to August 2001 in 64 hospitals and clinics. A SVR was obtained in 2515 patients (32.6%), among whom HCC was detected in 42 (1.7%) from 24 hospitals (38%).

In the second data collection, clinical data were received for 41 patients from 23 hospitals. Of these patients, three were excluded from the analysis because of detection of HCC within 1 year after IFN therapy (one patient), concomitant hepatitis B virus infection (one patient), and a history of treatment for HCC before IFN therapy (one patient). Accordingly, the study subjects comprised 38 patients who had developed HCC after SVR to IFN therapy for chronic hepatitis C. The profiles of the patients are shown in Fig. 1.

Table 1 summarizes the clinical features of the 38 HCV patients in whom HCC developed after SVR to IFN therapy. All of the patients were HCV RNA negative at the time of HCC detection, when their median age was 64 (range 38–77) years, and 34 of the patients (89%) were ≥60 years of age. Thirty-four patients (89%) were men (sex ratio 8.5:1). When data from before IFN therapy and at the detection of HCC were compared, there were significant improvements in platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and indocyanine green retention rate at 15 min (ICG R_{15}). In the 16 patients who underwent liver biopsy before IFN therapy and at the time of HCC detection, serial changes in histological fibrosis staging and activity grade were observed (Fig. 2). Histological activity grade improved significantly after IFN therapy ($P = 0.004$). However, there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$).

With regard to the HCC that developed, 31 patients (82%) had a solitary tumor and 22 patients (57%) had a tumor <3 cm in diameter. The median period until the detection of HCC was 4.7 years (range 1.4–9.0 years), and there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy (Fig. 3). The median period of medical follow-up after the termination of IFN therapy was 3 months (range 0.5–57 months), and eight patients were not followed up for 1 year or more. The maximum tumor size in these patients (median 60 mm; range 40–150 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median 25 mm; range 12–51 mm) ($P = 0.002$). Of the 38 patients, 16 underwent hepatic resection for HCC.

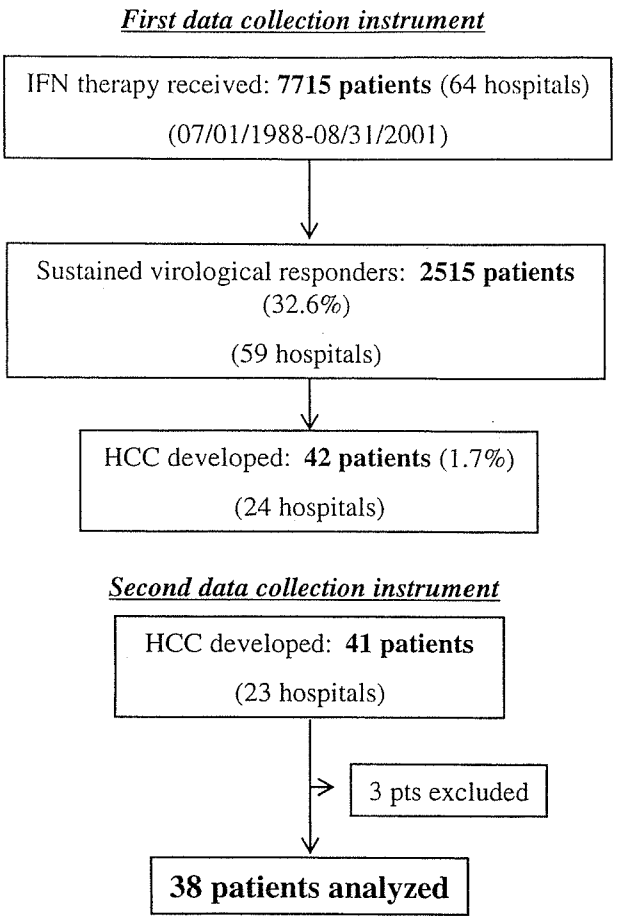

Figure 1 Profile of patients and data collection. One hospital did not respond to second data collection request. IFN, interferon; HCC, hepatocellular carcinoma.

DISCUSSION

Chronic hepatitis C is a progressive disease that is related to the development of cirrhosis and HCC. IFN, peginterferon, or combination therapy with ribavirin are widely used as standard treatments for chronic hepatitis C, the therapeutic scope being viral clearance and resolution of hepatic inflammation.[5–8] In theory, if successful in this respect, these treatments should have the additional effect of preventing HCC. Sustained eradication of HCV by IFN therapy has been shown to improve hepatic fibrosis as well as hepatic inflammation, and to suppress the occurrence of HCC.[5–15] However, there have been several reported cases of HCC that developed after successful IFN therapy.[11–27] The clinical features of HCC and the mechanisms of carcinogenesis have not yet been fully elucidated because development of HCC is very rare in sustained responders to IFN therapy.[20–27] Therefore, a multicenter study was set up to collect and analyze the clinical data for

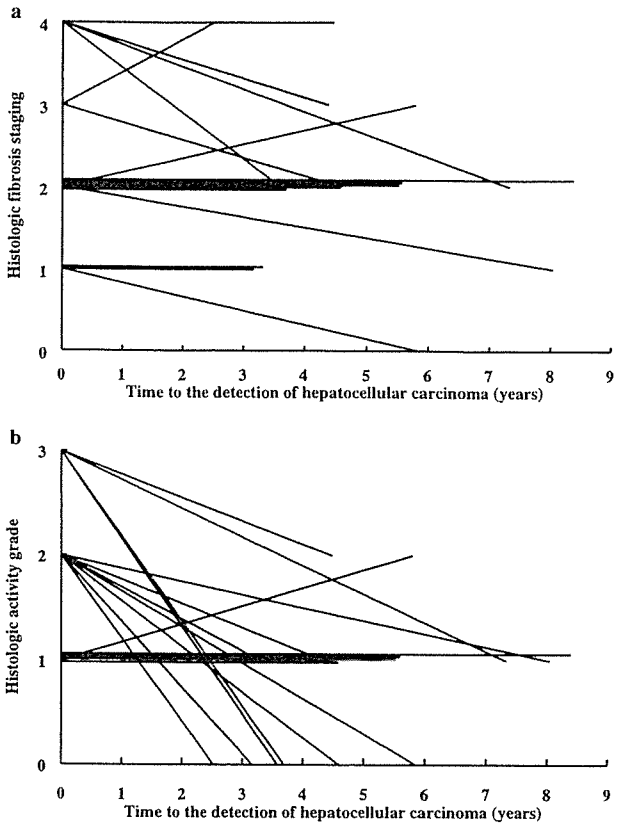

Figure 2 Serial changes in (a) histological fibrosis staging and (b) histological activity grading for each patient when compared before interferon therapy and at detection of hepatocellular carcinoma.

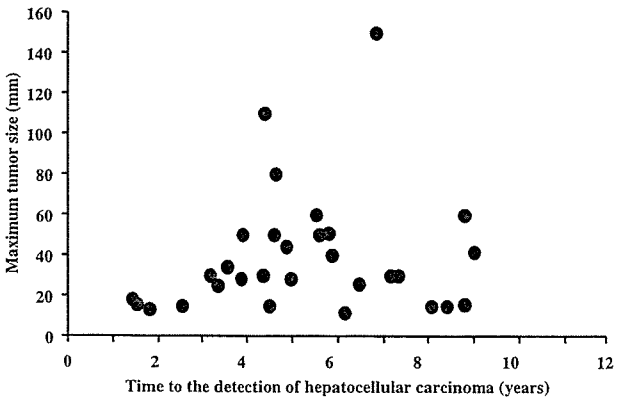

Figure 3 Maximum tumor size and time until detection of hepatocellular carcinoma.

patients who showed a SVR to IFN therapy for chronic hepatitis C and in whom HCC subsequently developed.

In this study, a total of 7715 patients with chronic hepatitis C received IFN therapy, and among them, a SVR was obtained in 2515 (32.6%). Among the patients with SVR who developed HCC, clinical data were collected for 38 patients. In regards to the clinical features of the HCC that developed in these patients, the percentage of those who were ≥60 years of age at the time of HCC detection (89%), and the percentage of men (89%) (sex ratio 8.5:1) were both high. In these patients, platelet count, albumin, AST, ALT, indocyanine green R_{15} and histological activity grade also improved significantly after IFN therapy ($P < 0.05$), although there was no significant improvement of histological fibrosis staging after IFN therapy ($P = 0.10$). Therefore, it was obvious that IFN therapy improved hepatic inflammation and hepatic function, as suggested by the results of other studies.[7–15] However, the other clinical features could not be clarified in this study, because we had no data from controls with which to compare the clinical variables of HCC that developed in patients showing SVR to IFN therapy. Potential control groups might include HCV patients with HCC who did not receive IFN therapy, or HCV patients with HCC who received IFN therapy but did not show a sustained response.[20–23] Additional comparative studies will be required in order to sufficiently elucidate the clinical features of HCC developing after SVR to IFN.

In the present study, there were 38 patients who developed HCC after successful IFN therapy, with a median period of 4.7 years (range 1.4–9.0 years) until detection of HCC. Moreover, the maximum tumor size in patients without medical follow-up for 1 year or more (median 60 mm) was significantly larger than in patients who were periodically followed up for 6 months or less (median 25 mm) ($P = 0.002$). As other studies have also indicated,[20,21] these findings suggest that the risk of HCC in sustained responders is not completely eliminated and that careful medical follow-up is important even after successful IFN therapy, which makes it difficult to determine the optimal follow-up period after SVR. If HCC had been detected at an earlier stage by regular follow-up, these patients could have been offered potentially curative treatment such as hepatic resection; such patients generally have good hepatic function after elimination of HCV. Moreover, it has also been reported that recurrence after curative treatment of HCC in SVR patients is less frequent than in non-SVR patients.[22,23] However, the enormous health care costs associated with screening all SVR patients for many years should be borne in mind. Therefore, it is also essential to identify the risk factors for development of HCC[20] and to establish the follow-up strategies in SVR patients.

Why does HCC develop even in patients showing a SVR to IFN therapy? HCV is a positive, single-stranded RNA virus without a DNA intermediate in its replicative cycle, so that integration of HCV nucleic acid sequences into the host genome, like that occurring in HBV infection, seems unlikely.[29] Therefore, HCV itself is probably not the causative factor of HCC after SVR. One assumption is that preexisting microscopic tumor foci that are not detected by diagnostic imaging are responsible for the appearance of HCC after SVR to IFN therapy, although in this study patients were excluded if HCC was detected within 1 year after the termination of IFN therapy. However, in the present series, there were nine patients in whom HCC less than 3 cm in size developed more than 5 years after IFN therapy. Although the rapidity of tumor growth may depend on individual tumor characteristics, considering

the late onset of small HCC in these patients, de novo HCC development after eradication of HCV should not be ignored. This has also been reported by Toyoda *et al.* on the basis of analysis that calculated the doubling time of HCC that occurred after SVR[24] and a long-term follow-up study of SVR patients.[21] It is conceivable that long-standing chronic liver inflammation and liver regeneration may provide the basis for tumor development. Carcinogenesis may not be a single-step event, but a complex, multi-step process, although the mechanisms are still unknown. Future studies should be aimed at defining the basic oncogenic mechanisms by which SVR patients develop HCC. Moreover, exploring the underlying mechanisms for the development of HCC in SVR patients may help identify new strategies for prevention of HCC.

In conclusion, even patients showing a SVR to IFN treatment of chronic hepatitis C and in whom hepatic function improves have the potential to develop HCC. The results of this study underline the importance of periodic medical follow-up for these patients.

ACKNOWLEDGMENTS

The authors thank Dr Michitami Yano for suggestions and support of this study, and Ms Mayuko Ikeda and Yuriko Kawaguchi for database development and manuscript preparation.

This study was supported in part by the Japanese Society of Gastroenterology, Kyushu Division, as a new project for 2001.

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APPENDIX I

Participating hospitals and clinics

In addition to the hospitals of the study authors, data were supplied by the following hospitals and clinics in the Kyushu Division of the Japanese Society of Gastroenterology: Nippon Steel Yawata Memorial Hospital; Yame General Hospital; 1st Department of Internal Medicine, Ryukyu University School of Medicine; 2nd Department of Internal Medicine, Kagoshima University School of Medicine; Hayato Town Medical Association Medical Center; Department of Internal Medicine, Saga University School of Medicine; Department of Medicine and Biosystemic Science, Kyushu University School of Medicine; Nishinihon Hospital; Kagoshima Kouseiren Hospital; Miyata Memorial Hospital; 2nd Department of Internal Medicine, Nagasaki University School of Medicine; Yonabaru Central Hospital; 2nd Department of Internal Medicine, Miyazaki University School of Medicine; Fukuoka Red Cross Hospital; Kita-kyushu Medical Center Hospital; Munakata Medical Association Hospital; Naika Yamaguchi Urban Clinic; Yamaga Municipal General Hospital; Shin Kokura Hospital; Iizuka Hospital; Oita Prefectural Hospital; 3rd Department of Internal Medicine, University of Occupational and Environmental Health School of Medicine; Kokura National Hospital; Kumamoto Regional Medical Center; Shin Beppu Hospital; Oita National Hospital; Kami Goto Hospital; 1st Department of Internal Medicine, Oita University School of Medicine; National Kyushu Medical Center; 1st Department of Internal Medicine, Nagasaki University School of Medicine; Hirahara Naika Clinic; Kumamoto National Hospital; Saiseikai Fukuoka General Hospital; Arita Ichou Hospital; Labour Welfare Corporation Moji Rosai Hospital; 1st Department of Internal Medicine, Kumamoto University; Kumamoto Red Cross Hospital; National Kyushu Cancer Center Hospital; Kagoshima Kyousaikai Nanpuh Hospital; Iwao Hospital; Saga Social Insurance Hospital; Labour Welfare Corporation Nagasaki Rosai Hospital; Oita Red Cross Hospital; Koebaru Central Hospital; National Sanatorium Kumamoto South Hospital; 2nd Department of Internal Medicine, Oita Medical University; Labour Welfare Corporation Kumamoto Rosai Hospital; Amakusa Regional Medical Center; Miyuki Hospital; Saiseikai Futsukaichi Hospital; Hakujyuji Hospital; Hakuaikai Hospital.

Antibody to hepatitis B core antigen is associated with the development of hepatocellular carcinoma in hepatitis C virus-infected persons: A 12-year prospective study

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Received December 9, 2005; Accepted January 26, 2006

Abstract. Several studies have reported that antibody to hepatitis B core antigen (anti-HBc) positivity may influence the development of hepatocellular carcinoma (HCC) in chronic hepatitis C patients, but the evidence is still not conclusive. In this study, we examined whether the presence of anti-HBc positive was associated with the development of HCC in hepatitis C virus (HCV)-infected subjects among the residents in an HCV hyperepidemic area who were followed up for 12 years. In an HCV hyperendemic area (positive rate of anti-HCV: 23.4%), 509 residents were examined by health screening in 1990. After 12 years of follow-up, we evaluated the risk factors for HCC. The incidence of HCC was compared between anti-HBc positive and anti-HBc negative subjects after 12 years of prospective observation. Univariate and multivariate analyses were conducted to determine risk factors for the development of HCC. The incidence of HCC was significantly higher in the anti-HBc positive group (13 subjects) than in the anti-HBc negative group (0 subjects) (P=0.012). Multivariate analysis identified positivity for anti-HBc and HCV RNA, history of icterus, and female gender as independent determinants of the development of HCC. Our findings provide clear evidence in a prospective study that presence of anti-HBc, that is, past hepatitis B virus (HBV) infection, is a risk factor for the development of HCC in HCV-infected people.

Introduction

The number of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection carriers worldwide is estimated at 350 million (1) and 170 million (2), respectively. HBV and HCV infections include substantial proportions of cases with past infection, asymptomatic carriers, acute hepatitis and chronic hepatitis, and HBV infections may cause fulminant hepatitis. Especially, chronic HBV and HCV infections may lead to cirrhosis and hepatocellular carcinoma (HCC) (1,3). It was reported that the frequency of HCC due to chronic HCV infection is higher in Japan than in any other country (4). Several studies have reported that occult HBV infection may also be one of the causative factors of HCC (5,6). The presence of occult HBV infection is diagnosed based on the fact that HBV DNA still exists in serum and liver tissue after hepatitis B surface antigen (HBsAg) disappears in acute or chronic HBV infection (7-9), or even after antiviral treatment is successful. Although some studies reported that occult HBV infection is associated with HCV-related liver dysfunction (10) or the development of HCC (11-13), these associations have still not been clearly demonstrated in a prospective study.

A higher incidence of HBV DNA is commonly seen in patients with anti-HBc-positive serology than in those with anti-HBc negative serology in coinfections with HBV and HCV (10), and using PCR amplification, most studies have demonstrated the presence of the HBV DNA genome in 22% to 87% of the patients who are HBsAg negative and HCV RNA positive (10,14-18). Some studies showed that HBV infection could occur in recipients of livers donated from subjects with anti-HBc but without HBsAg (19,20). That is, anti-HBc, which was initially considered to be an index for the past HBV infection in which all HBV had been cleared, has emerged as a convincing marker of occult hepatitis B (19,21-23). Also, several studies showed that the anti-HBc positivity was associated with the development of HCC in patients with HCV-associated chronic liver disease (11,24-26), but these associations have not been clearly demonstrated.

Since 1990, we have conducted health screenings of the residents of H town (adult population: 7,389), Fukuoka prefecture in northern Kyushu, Japan (27). This town is known for its high prevalence of liver disease. We previously reported that the town had a high prevalence of HCV carriers, 120/509 (23.6%) in 1990, and that HCV infection was the principal cause of liver dysfunction and HCC (27,28).

In the present study, we analyzed the influence of anti-HBc positivity on the development of HCC in HCV-infected people in the same town during 12 years in a prospective manner.

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Key words: antibody to hepatitis B core antigen, occult hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, HCV hyper epidemic area

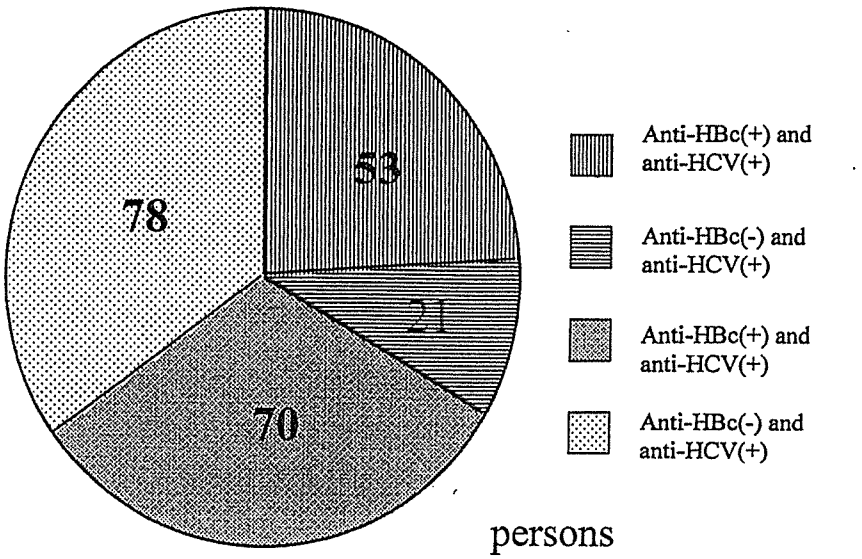

Figure 1. Diagram showing incident of hepatitis virus markers (anti-HCV and anti-HBc) among the 222 inhabitants 12 years ago. Fifty-three inhabitants were anti-HBc positive and anti-HCVpositive, 21 were anti-HBc negative and anti-HCVpositive, 70 were anti-HBc positive and anti-HCV negative, and 78 were anti-HBc negative and anti-HCV negative.

Subjects and methods

Subjects. In 1990, of a total 9,799 inhabitants, 739 (10%) of the 7,389 inhabitants >20 years old were randomly selected as follows: the names of the residents (as they appeared on their resident cards) were arranged in order according to the Japanese phonetic syllabary. Then every tenth resident was selected. As a result, 509 subjects (6.9% of H town residents) gave their informed consent to participate in the study.

Of 509 participants initially screened in 1990, 69 people had died and 55 people had moved to other regions as of 2002. Thus, 385 of the original 509 residents survived in the area and 139 residents agreed to participate in the medical follow-up survey, while 26 did not agree to participate, and the remaining 220 residents did not declare their intention either way in 2002. For 14 of these remaining 220 inhabitants, the records were obtained from the primary physicians. Consequently, we analyzed the outcome in terms of the liver disease in 222 inhabitants (69+139+14) in 2002.

Information on cigarette smoking, alcohol consumption, and history of icterus, and blood transfusion was obtained at the time of enrollment through interviews by the doctors in charge and experienced public health nurses. Smoking was defined as >10 cigarettes per day for >10 years. Alcohol consumption was defined as a daily intake of ≥75 g of ethanol per day for >10 years.

Serological assay. In 1990, sera were collected from all the participants, and conventional liver function tests were performed: serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transpeptidase (γ-GTP), total protein (TP), albumin (Alb), total cholesterol (TC), total bilirubin (TB), zinc turbidity (ZTT) were measured. Anti-HCV was measured using HCV PHA 2nd generation kits (Dainabot Co. Ltd., Tokyo, Japan). These results were confirmed using a second generation recombinant immunoblot assay (RIBA II) (Ortho Diagnostic System, Tokyo, Japan). Measurement of HBsAg and anti-HBc was performed with an enzyme immunoassay kit (Mizuho Medy Co. Ltd., Tosu, Saga, Japan). Titers of anti-HBc yielding >70% inhibition were assessed as positive.

Detection of HCV RNA by RT-PCR. All subjects who were anti-HCV-positive were tested for the presence of serum HCV RNA, which was detected by reverse transcription-nested polymerase chain reaction (RT-nested PCR) using primers based on the sequences of the 5'UTR (untranslated region) of the HCV genome, as described previously (29).

Statistical analysis. Continuous data were expressed as mean ± SD, minimum and maximum. Categorical data were expressed as frequency and/or percentage. For comparing the background between anti-HBc positive and negative, the χ^2 and Wilcoxon's test were used to analyze quantitative data. Univariate and multivariate analysis were performed by logistic regression to calculate odds ratio and its 95% confidence interval. The SAS (statistical analysis system) computer program (release 8.2; SAS Institute Inc., Cary, NC, USA) was used for the logistic regression. A P-value of <0.05 was considered statistically significant.

Results

In 2002, anti-HCV was detected in 74 of the 222 inhabitants (Fig. 1). HCV RNA was detected in 53 (71.6%), HBsAg in 1 (1.4%), and anti-HBc in 53 (71.6%) of these 74 people. We asked the primary physician of these 74 inhabitants about the diagnosis of liver disease, and found thereby that 8 inhabitants had died of HCC and 5 inhabitants had been treated for HCC (total 13 inhabitants).

The 74 inhabitants were divided into two groups: 53 who were positive and 21 who were negative for anti-HBc, and the clinical characteristics observed in the screening were compared between the two groups. No significant differences

Table I. Characteristics of anti-HCV positive patients with and without anti-HBc.

Characteristics	Anti-HBc positive (N=53)	Anti-HBc negative (N=21)	P-value
Age (year)	62.3±10.9	58.0±16.4	NS
Sex: M:F	23:30	05:16	NS
Smoking (%)	16 (30.2)	4 (19.0)	NS
History of icterus (%)	8 (15.1)	3 (14.3)	NS
Alcohol consumption (%)	3 (5.7)	2 (9.5)	NS
History of blood transfusion (%)	8 (15.1)	4 (19.0)	NS
ALT level (IU/l)	40.6±30.8	27.5±17.9	NS
HBsAg (%)	1 (1.9)	0 (0)	NS
HCV RNA (%)	39 (73.6)	14 (66.7)	NS
HCC (%)	13 (24.5)	0 (0)	0.012

Age and serum ALT level were expressed as mean ± SD. HCC, hepatocellular carcinoma; NS, not significant.

Table II. Univariate analysis of risk factors that influence the development of HCC.

Factors	HCC group (n=13)	non-HCC group (n=61)	Odds ratio	95% CI	P-value
Age (years)	65.3±8.1 (53-82)	60.1±13.4 (23-89)	1.035	0.984-1.088	0.1866
Sex: male (%)	6 (46.2)	22 (36.1)	0.658	0.196-2.205	0.4976
Smoking (%)	4 (30.8)	13 (21.3)	1.641	0.435-6.190	0.4646
Alcohol consumption (%)	5 (38.5)	22 (36.1)	1.108	0.323-3.804	0.8706
History of blood transfusion (%)	3 (23.1)	8 (13.1)	1.988	0.448-8.810	0.3659
History of icterus (%)	5 (38.5)	5 (8.2)	7.000	1.652-29.667	0.0083[a]
AST (IU/l)	65.5±31.1 (28-131)	33.0±21.9 (13-132)	1.041	1.015-1.068	0.0016[a]
ALT (IU/l)	57.5±24.8 (20-108)	32.6±27.1 (9-160)	1.028	1.006-1.050	0.0119[a]
γ-GTP (IU/l)	127.1±195.3 (17-720)	32.4±34.2 (7-196)	1.015	1.003-1.027	0.0158[a]
Total protein (IU/l)	7.97±0.88 (6.6-10.0)	8.05±0.58 (6.6-9.8)	0.808	0.309-2.107	0.6622
Albumin (g/dl)	3.98±0.49 (3.0-4.9)	4.33±0.31 (3.2-4.8)	0.094	0.017-0.507	0.0060[a]
Total cholesterol (mg/dl)	160.5±33.1 (111-224)	173.8±32.5 (111-257)	0.987	0.967-1.007	0.1851
Total bilirubin (mg/dl)	1.01±0.50 (0.5-2.3)	0.77±0.27 (0.4-1.3)	7.537	1.170-48.533	0.0335[a]
ZTT (KU)	15.35±5.76 (1.1-21.7)	11.40±4.86 (2.5-27.4)	1.161	1.026-1.314	0.0183[a]
Anti-HBc (%)	13 (100)	40 (65.6)	9.150	1.407-	0.0161[a]
HCV RNA (%)	13 (100)	40 (65.6)	9.150	1.407-	0.0161[a]

[a]P<0.05; HCC, hepatocellular carcinoma; CI, confidence interval. Age, AST, ALT, γ-GTP, total protein, albumin, total bilirubin and ZTT were expressed as mean ± SD (range).

were observed between the two groups in age, sex, smoking, history of icterus or blood transfusion, alcohol consumption, ALT level, HBsAg, or HCV RNA. Significant differences were observed for the incidence of HCC (13 versus 0) between these two groups (P=0.012) (Table I).

Univariate and multivariate analyses of factors that influenced the incidence of HCC. The influence of age, sex, smoking, history of icterus, history of blood transfusion, alcohol consumption, AST, ALT, γ-GTP, TP, Alb, TC, TB, ZTT, anti-HBc and HCV RNA on the development of HCC was analyzed by univariate and multivariate analyses.

Table II shows the basic characteristics of the 74 inhabitants with anti-HCV divided into two groups: a group with HCC (HCC group) and a non-HCC group, and shows the results of univariate analyses. The mean age and sex were not significantly different between the HCC group and non-HCC group. Serum levels of AST, ALT, γ-GTP, TB, and ZTT were significantly higher in the HCC group than in the non-HCC group (P<0.05). The serum level of Alb was significantly lower in the HCC group than in the non-HCC group (P<0.05). The frequency of anti-HBc, HCV RNA, and history of icterus were significantly higher in the HCC group than in the non-HCC group (P<0.05). The frequency of smoking, alcohol

Table III. Multivariate analysis of risk factors that influence the development of HCC.

Factors	Odds ratio	95% CI	P-value
Age (years)	0.987	0.852-1.132	0.8428
Sex: female	190.517	2.157- >999.999	0.0188[a]
Smoking	40.580	0.656- >999.999	0.0824
Alcohol consumption	5.051	0.163-3.804	0.3644
History of blood transfusion	0.964	<0.001- >999.999	0.9918
History of icterus	311.186	5.066- >999.999	0.0042[a]
AST (IU/l)	1.013	0.855-1.244	0.8776
ALT (IU/l)	0.974	0.791-1.101	0.7013
γ-GTP (IU/l)	1.006	0.990-1.080	0.6950
Total protein (IU/l)	15.131	0.227- >999.999	0.2035
Albumin (g/dl)	<0.001	<0.001-11.319	0.1236
Total cholesterol (mg/dl)	1.018	0.952-1.106	0.6028
Total bilirubin (mg/dl)	7.911	0.060- >999.999	0.4127
ZTT (KU)	0.695	0.370-1.196	0.1853
Anti-HBc positive	>999.999	1.556-	0.0292[a]
HCV RNA positive	>999.999	3.767-	0.0063[a]

[a]P<0.05; HCC, hepatocellular carcinoma; CI, confidence interval.

consumption, and history of blood transfusion were not significantly different between the HCC group and non-HCC group.

Multivariate logistic regression analyses identified anti-HBc positivity, HCV RNA positivity, history of icterus, and female sex as independent risk factors for the development of HCC (Table III).

Discussion

Several studies have shown that anti-HBc positivity was associated with the development of HCC in patients with HCV-associated chronic liver disease (11,24-26). However, considering the natural history of all HCV infections, the results of those previous studies have some problems, i.e., the observation period was short and the research was performed in a retrospective manner in patients with chronic hepatitis and liver cirrhosis. Our study was a prospective study that investigated the disease progress after 12 years, and was thought to reflect the natural history of HCV infections, because we did not investigate only HCV-associated chronic liver disease but also covered all HCV infections such as past HCV infection and asymptomatic carriers of HCV (30,31). In this study, we obtained clear evidence that anti-HBc-positivity was a risk factor for the development of HCC in HCV-infected people.

It has been suggested that HBV can induce liver tumor formation by at least two distinct mechanisms. First, HBV DNA sequences are frequently found integrated into chromosomes of hepatocytes that have evolved into HCC, and a direct role of HBV in hepatocarcinogenesis has thus been inferred (32,33). Second, HBV DNA sequences may be caused by disruption of tumor suppressor gene function (34). It has been shown that HBV DNA sequences can be detected in some of the liver or serum from anti-HBc-positive patients (9,10), and the presence of anti-HBc does not entirely exclude the possibility of chronic HBV infection. Though the presence of anti-HBc has been used as a marker of past HBV infection, the integration of HBV DNA in hepatocytes may cause carcinogenesis, as noted above. That is, anti-HBc-positivity may represent occult HBV infection. The presence of anti-HBc alone, in the absence of HBV DNA testing, has been used in some studies as a marker of occult hepatitis B (19,21-23). Pollicino *et al* provided clear evidence that occult HBV was a risk factor for the development of HCC and showed that the potential mechanisms whereby HBV might induce tumor formation occur in most cases of occult infection (6).

To detect occult HBV infection, it is necessary to examine whether HBV DNA is present. However, serum HBV DNA levels are frequently below the limits of detection in anti-HBc-positive patients, and there is a pronounced risk of false-positive results from contamination (35) or amplification of non-HBV-DNA targets, and the sensitivity of detection is variable (36,37). In a previous study in which serum HBV DNA was tested in 20 anti-HBc positive patients with HCV-associated HCC, HBV DNA was not detected by a real-time PCR assay with a minimum detection limit of $10^{1.7}$ copies/ml (1.7 log copies/ml) (38,39). Considering these results, it might not be possible to detect serum HBV DNA in some anti-HBc-positive subjects. Therefore, if we could examine liver tissues by PCR to examine whether occult HBV infection is present, we could be more certain of the presence of occult HBV infection.

In contrast to our findings, in some studies anti-HBc positivity was not found to be associated with the development

of HCC in patients with HCV-associated chronic liver disease (9,39,40). One study showed that anti-HBc was detected significantly more frequently in blood donors with than without anti-HCV, but the prevalence of anti-HBc was no different between the patients with HCV-associated HCC and anti-HCV-positive blood donors. Therefore, no epidemiological evidence was obtained for a role of past HBV infection in hepatocarcinogenesis in patients infected with HCV in Japan (40). Also, Yano *et al* showed that the clinical features of HCV-associated HCC were unaffected by anti-HBc-positivity (39). In addition, a study in Taiwan suggested that occult HBV infection might have little influence on the clinicopathologic course of chronic HCV infection (9).

It was reported that the frequency of HCC due to chronic HCV infection is higher in Japan compared with any other country (4). If the frequency of HCC due to chronic HCV infection is high, it is necessary to consider the possibility that anti-HBc positivity may be associated with hepatocarcinogenesis. In addition to HBV, other environmental and host factors might also be associated with the pathogenesis of HCC (4,41-43).

We continued carrying out health screenings of the residents of H town and conducted a cohort study of liver disease among the same residents over a 12-year period. The results of this study showed that anti-HBc is associated with the development of HCC in HCV-infected people.

Acknowledgements

We would like to thank the following institutions for collection of data of the residents' liver disease: Amagi Asakura Medical Association Asakura Hospital, Ishii Surgery Clinic, Shigematsu Medical Office, Tanabe Medical Office, Haki Clinic, Fukuoka Prefectural Hepato-gastroenterological Center, Moriyama Medical Office, Yamaga Medical Office, Wada Surgery Clinic, Chikugo River Onsen Hospital, Harazuru Onsen Hospital, Digestive Tract Internal Medicine of St. Mary's Hospital, Digestive Tract Internal Medicine of Koga Hospital, and Majima Digestive Tract Clinic. Moreover, we thank the following companies for supplying the related inspection reagent kits for the hepatitis virus: Ortho-Clinical Diagnostics, K.K., Roche Diagnostics K.K., and Abbott Laboratories. This study was supported in part by grants as a project for establishing new high technology research centers, by a Grant-in-Aid for Scientific Research (C) (No. 11670548), by a Grant-in-Aid for Encouragement of Young Scientists (No. 14770256) from the Ministry of Education, Science, Sports and Culture of Japan, and the Hepatitis C Research Group (2001-2003) under the auspices of the Ministry of Health, Labor and Welfare.

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日本アフェレシス学会雑誌 25(3):233-239, 2006

原著論文

C型慢性肝炎へのIFNα-2b・リバビリン療法における二重濾過血漿交換併用療法の臨床的検討

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Clinical Evaluation of Double Filtration Plasmapheresis and Interferon/Ribavirin Combination Therapy for Patients with Chronic Hepatitis C

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Summary Since double filtration plasmapheresis (DFPP) can reduce the hepatitis C virus (HCV) load in sera, DFPP and interferon/ribavirin combination therapy was clinically evaluated in patients with chronic hepatitis C. Six patients infected with HCV genotype 1 b and high viral loads were enrolled in this study. From the initiation of interferon/ribavirin therapy, DFPP was performed three times within one week. The duration of interferon/ribavirin therapy was 24 or 48 weeks. The serum viral load was reduced by the DFPP procedure. Finally, sustained virological responses were obtained in 5 patients (83.3%). No serious side effects were observed. In conclusion, DFPP and interferon/ribavirin combination therapy is judged to be effective in patients infected with HCV genotype 1 and high viral loads.

Key words: double filtration plasmapheresis, interferon, ribavirin, hepatitis C virus

要 旨 HCV粒子は，その直径が55～65 nmとされており，二重濾過血漿交換（double filtration plasmapheresis: DFPP）に使用する膜のpore sizeを30 nmにすることで，HCVを物理的に除去することが可能である．そこでC型慢性肝炎のGenotype 1b，高ウイルス量例（100 KIU/ml以上）の難治例に対し，インターフェロン・リバビリン（IFN・Rib）療法にDFPPを併用し治療効果を検討した．対象はC型慢性肝炎患者6例である．IFN・Rib療法は24週投与例3例，残り3例はIFN治療のみをさらに24週間追加し計48週投与した．DFPPは，IFN・Rib療法開始日から1週以内に計3回行った．二次膜を通過した血漿中のウイルス量は，全例検出限界以下となった．6例中5例で治療開始後8週目に血中HCV RNA陰性化が得られた．IFN治療期間24週の3例中2例が著効で，治療期間48週の3例中3例で著効が得られた．DFPPとIFN・Rib療法の併用は難治例のC型慢性肝炎に対し高い治療効果を有すると考えられた．

1. 緒 言

C型慢性肝炎のGenotype 1b，高ウイルス量例のいわゆる難治例に対しては，インターフェロン・リバビリン（IFN・Rib）24週間の併用療法が開発されたが，著効率は，20%前後であった[1]．最近はペグインターフェロン・リバビリン（PEG IFN・Rib）48週の併用療法が標準（2004年12月承認）となっており，著効率は約50%に上昇した[2]ものの未だ満足いくものではない．よってさらなる治療法の改善が求められている．

ところで二重濾過血漿交換療法（double filtration plasmapheresis: DFPP）は血漿中の蛋白を濾過することで全身性エリテマトーデス，高LDL血症など各種疾患の症状改善に効果を上げ，すでに臨床応用されている[3,4]．一方，C型肝炎ウイルス（HCV）は，そ

2006年4月27日受付，2006年6月30日受理．

の直径が55～65 nmとされており，DFPPに使用する膜のpore sizeを30 nmにすることで，HCVを物理的に除去できると考えられる．さらにHCVは，血中蛋白とくにLDL（low density lipoprotein）に結合していること[5,6]から，さらにその直径は大きくなり，DFPPを用いて，HCVをこの蛋白と一緒に血中から濾過し除去することが可能と考えられる．また金沢大学のSakai，Kanekoらは，血液中におけるHCVの存在様式がインターフェロン療法の治療効果と関連することを報告し[7]，吸着体による血液中のHCVの除去など，血中ウイルスを除去することによってインターフェロンの治療効果が増強する可能性について検討を行ってきた．現在，C型慢性肝炎患者に対して血漿交換療法（DFPP）を行いウイルス量を低下させると同時にインターフェロン療法を行うウイルス減量療法研究会が，横浜労災病院藤原研司総括医師を中心とした全国多施設臨床研究により検討中である．そこで今回，全国治験の一部の症例である当科の症例でGenotype 1b，高ウイルス量のC型慢性肝炎例におけるIFN・Rib療法にDFPPを併用し，ウイルス学的改善効果および安全性につき検討した．

2. 対象と方法

2.1 対　　象

対象は2003年9月から2004年9月までの間に当科にてIFN・RibおよびDFPP療法を受けたGenotype 1b，高ウイルス量（100 KIU/ml以上）のC型慢性肝炎6例である．DFPPの臨床試験は当大学での倫理委員会で承認を受け，患者には全員文書で同意を得た．また同時期にIFN・Rib療法のみを受け，治療が完遂できた14名を対照群とした．IFN・RibおよびDFPP併用群（以下DFPP群）と対照群の背景を表1に示した．年齢，臨床検査値は平均±標準偏差で表示した．DFPP群は，IFNの投与期間によりさらに2つのグループに分け，IFN投与期間が24週の症例をDFPP-1群とし3例（全例男性，平均年齢53.0±2.9歳）であり，IFN・Rib投与を24週間終了後IFNのみを24週間追加し計48週間治療を行った症例をDFPP-2群とし3例（全例男性，平均年齢56.7±6.3歳）であった．DFPP-2群は，患者の強い希望によりIFN治療を24週間追加した例である．DFPP-1，2群共に投与前のHCV-RNA量は，100以上500 KIU/ml未満が1例，500 KIU/ml以上が2例であった．

また，対照群は14例（男性8例，女性6例，平均年齢50.9±8.7歳）で，投与前のHCV RNA量は，100以上500 KIU/ml未満が6例，500 KIU/ml以上が8例であった．

両群共に，肝組織学的診断では新犬山分類によるF 4，A 3の症例はなかった．

2.2 IFN・Rib療法

IFN・Rib療法の方法は，我が国で行われている最も標準的な方法で行った．すなわちIFNα-2b，600万単位を2週連日投与後週3回計24～48週間投与した．Ribは体重60 kg以上の例では800 mg/日，60 kg未満の例では600 mg/日を計24週間投与した．

表1　背景因子

	項目	DFPP-1群	対照群	DFPP-2群
治療期間（週）	IFN	24	24	48
	リバビリン	24	24	24
性別	男	3	8	3
	女	0	6	0
年齢（歳）		53±2.9	50.9±8.7	56.7±6.3
肝生検（Staging）	0/1/2/3/4	0/1/2/0/0	0/3/4/4/0	1/1/1/0/0
肝組織（Grading）	0/1/2/3	0/1/2/0	0/2/9/0	0/3/0/0
肝生検	不明	0	3	0
過去のIFN治療歴	無し	2	13	2
	有り	1	1	1
	不明	0	0	0
HCV RNA量（KIU/ml）	100以上500未満	1	6	1
	500以上	2（66.7%）	8（57.1%）	2（66.7%）
ALT値（IU/L）		77.3±17.6	114.1±63.4	113.3±99
血小板数（$10^4/\mu l$）		18.8±4.1	13.9±4	16.3±2.3
フィブリノーゲン（mg/dl）		218.7±31.7	—	198.7±19.4

2.3 DFPP療法

DFPPはIFN・Rib療法開始日から1週以内に，開始日，第2日，第4日を原則とし計3回行ったが，日祭日や患者希望などにより一部の症例で第1，3，5日などとした．DFPPには，血漿分離器としてOP-08Wを，血漿成分分離器としてEC-50Wを用い，血漿処理量は1回3,000 mlとした．血中のHCV RNA量は，アンプリコアモニター法version 2.0（ロシュダイアグノスティックス(株)，東京）を用い，第1日目はDFPP直前，DFPP施行中の1，2時間目およびDFPP終了直後に，それ以降は7日間毎朝測定し，DFPP施行日はDFPP直後にも測定した．その後は2週目，4週目に，以降は1カ月毎に測定した．HCV RNA定性はアンプリコア法（ロシュダイアグノスティックス(株)，東京）を用いた．なおDFPPによりウイルスが濾過・除去されていることを確認するために，濾過膜（二次膜）前後でHCV RNA量を測定した．またAST値，ALT値，フィブリノーゲン値，検血一般血液一般検査も第1週目は毎朝採血し，以降は2週目，4週目，1カ月毎に測定した．またフィブリノーゲン値が100 mg/dl未満となった場合はその日のDFPPを中止とし，翌朝フィブリノーゲン値の回復を確認してDFPPを行うこととした．

2.4 治療効果判定

IFN・Rib療法（およびDFPP併用）の最終的な治療効果判定は，IFN療法終了後6カ月以上血中HCV RNA定性が持続陰性の例を著効，それ以外を無効とした．

3. 結　　果

3.1 DFPPの二次膜前後でのウイルス除去効果

DFPP群は全例とも目標血漿処理量である3,000 mlの処理を行うことができた．血漿処理は3〜6時間必要であった．血漿処理途中の時点におけるDFPP装置の二次膜直前の血漿中HCV RNA量は，1,113.3±840.8 KIU/mlであったが，二次膜処理後は全例検出限界以下となり，定性検査でも陰性であることを確認した．さらに，血漿処理の終了時における二次膜直前の血漿中ウイルス量は，995.0±1,089.3 KIU/mlであったが，二次膜処理後は同様に全例検出限界以下となり，定性検査でも陰性であった．以上より，DFPPの二次膜での血漿中ウイルス除去効果が確認された．

3.2 ウイルス血症改善度

3.2.1 ウイルス陰性化率の推移

投与中および投与終了24週後までの血中HCV RNA定量または定性の成績を表2，3に示した．また，DFPP-1および2群，対照群の投与開始4，8，

表2　DFPP-1群（IFN 24週）と対照群のHCV RNAの推移

	Patient No.	HCV RNA量 (KIU/ml)	IFN治療歴	IFN・リバビリン24週投与							投与終了後観察期間						判定
				2W	4W	8W	12W	16W	20W	24W	4W	8W	12W	16W	20W	24W	
DFPP-1群	1	460	無し	<0.5	(−)	(−)	(−)	NT	NT	(−)	(−)	(−)	(−)	(−)	NT	(−)	著効
	2	980	無し	0.8	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	NT	NT	(−)	著効
	3	1,900	有り（無効）	<5	(+)	(−)	(−)	(−)	NT	(−)	(−)	NT	(+)	(+)	(+)	(+)	無効
対照群	4	197	無し	(+)	(+)	(+)	(−)	NT	NT	11	(+)	(+)	(+)	(+)	(+)	18	無効
	5	435	無し	(+)	(+)	(+)	(+)	(−)	NT	20	(+)	(+)	36	(+)	(+)	45	無効
	6	240	無し	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	著効
	7	380	無し	13	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	98	(+)	(+)	34	無効
	8	404	有り	(+)	(+)	(+)	(+)	(+)	(+)	67	(+)	(+)	(+)	(+)	(+)	71	無効
	9	234	無し	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	著効
	10	599	無し	1	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	著効
	11	>850	無し	(+)	(+)	(+)	(−)	NT	NT	NT	NT	(−)	NT	NT	NT	NT	無効
	12	>850	無し	45	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	無効
	13	846	無し	137	(+)	(+)	(+)	(+)	(−)	14	(+)	(+)	102	(+)	(+)	56	無効
	14	>850	無し	(+)	(−)	(−)	(−)	NT	NT	22	(+)	(+)	215	(+)	(+)	61	無効
	15	745	無し	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	無効
	16	505	無し	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	無効
	17	>850	無し	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	無効

表中の数字は，アンプリコアモニター法によるHCV RNA量を示す（KIU/ml），(+)：HCV RNA定性陽性，(−)：HCV RNA定性陰性．NT：not tested.

表3　DFPP-2群（IFN 48週）のHCV RNAの推移

	Patient No.	HCV RNA量 (KIU/ml)	IFN治療歴	IFN・リバビリン24週投与 2W	4W	8W	12W	16W	20W	24W	IFN 24週単独投与 28W	32W	36W	40W	44W	48W	投与終了後観察期間 4W	8W	12W	16W	20W	24W	判定
DFPP-2群	1	650	無し	<5 (+)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	著効
	2	650	有り (無効)	0.7	2	(−)	(−)	(+)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	(−)	著効
	3	1,300	無し	30	NT	(+)	(−)	NT	(−)	(−)	NT	(−)	(−)	(−)	NT	(−)	NT	NT	(−)	NT	NT	(−)	著効

表中の数字は，アンプリコアモニター法によるHCV RNA量を示す(KIU/ml)，(+)：HCV RNA定性陽性，(−)：HCV RNA定性陰性．NT：not tested.

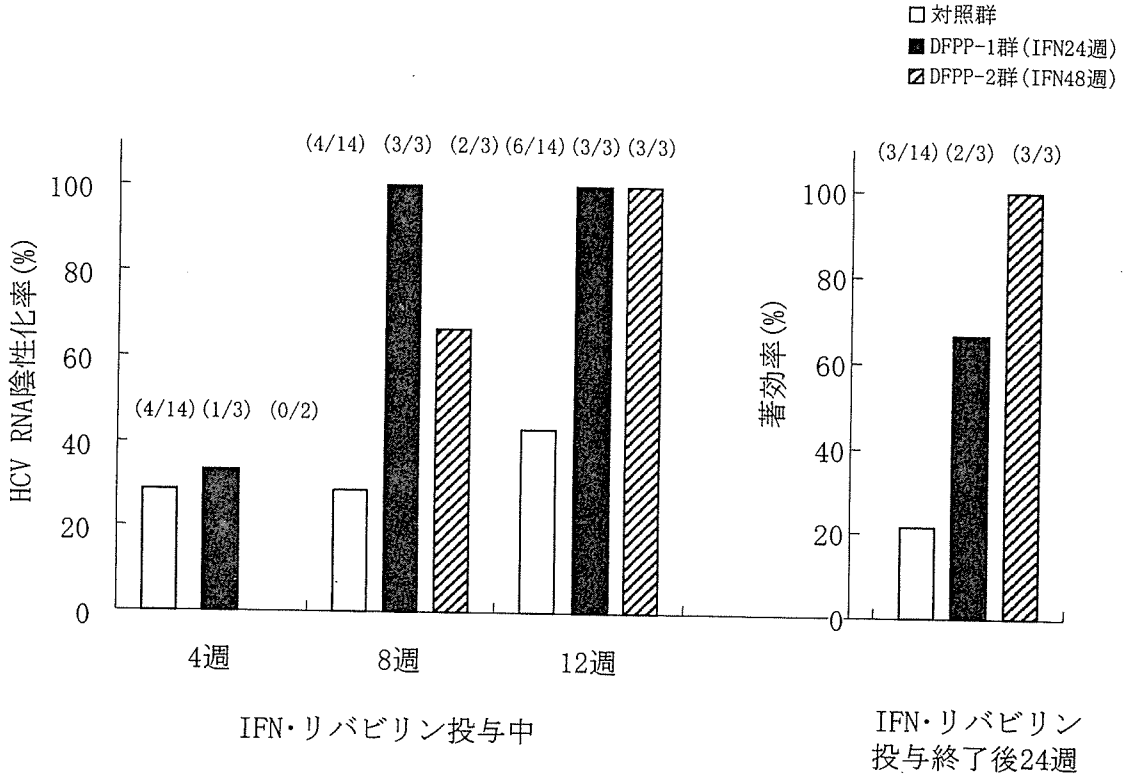

図1　HCV-RNA陰性化率の推移と著効率

左図：IFN・リバビリン投与中の4，8，12週目のHCV RNA定性陰性化率を示す．グラフ上部の括弧内は，症例数を示す．DFPP群は対照群に比し8，12週目の陰性化率が高い．

右図：IFN・リバビリン投与終了後24週目での著効率（HCV RNA陰性化率）を示す．グラフ上部の括弧内は，症例数を示す．DFPP群は対照群に比し著効率が高い．

12週後のHCV RNA定性陰性化率，さらに著効率を図1に示した．

DFPP-1群では，治療開始2週目において3例中2例で検出限界以下となり，8週目で3例（100%）にHCV RNAの陰性化が認められた．一方，対照群は8週目で，14例中4例（28.6%）に陰性化が認められた．治療開始12週目においてもDFPP-1群は3例共（100%）に陰性化を示したが，対照群では14例中6例（42.9%）の陰性化であった．最終的な著効率は，DFPP-1群で66.7%（3例中2例）であり，対照群は，21.4%（14例中3例）であった．

DFPP-2群では，治療開始2週目において3例中1例で検出限界以下となり，1例は0.7 KIU/mlまでHCV RNA量の低下が確認された．8週目で2例（66.7%）にHCV RNAの陰性化が認められ，治療開始12週目では3例共に陰性化を示した．著効率は，100%（3例中3例）であった．

3.2.2　IFN・RibおよびDFPP併用例のHCV RNA量の動態について

DFPP-1群症例No.1のHCV RNA量の動態について図2に示した．DFPP治療開始前にはHCV RNA量460 KIU/mlであったが，1回目のDFPPの

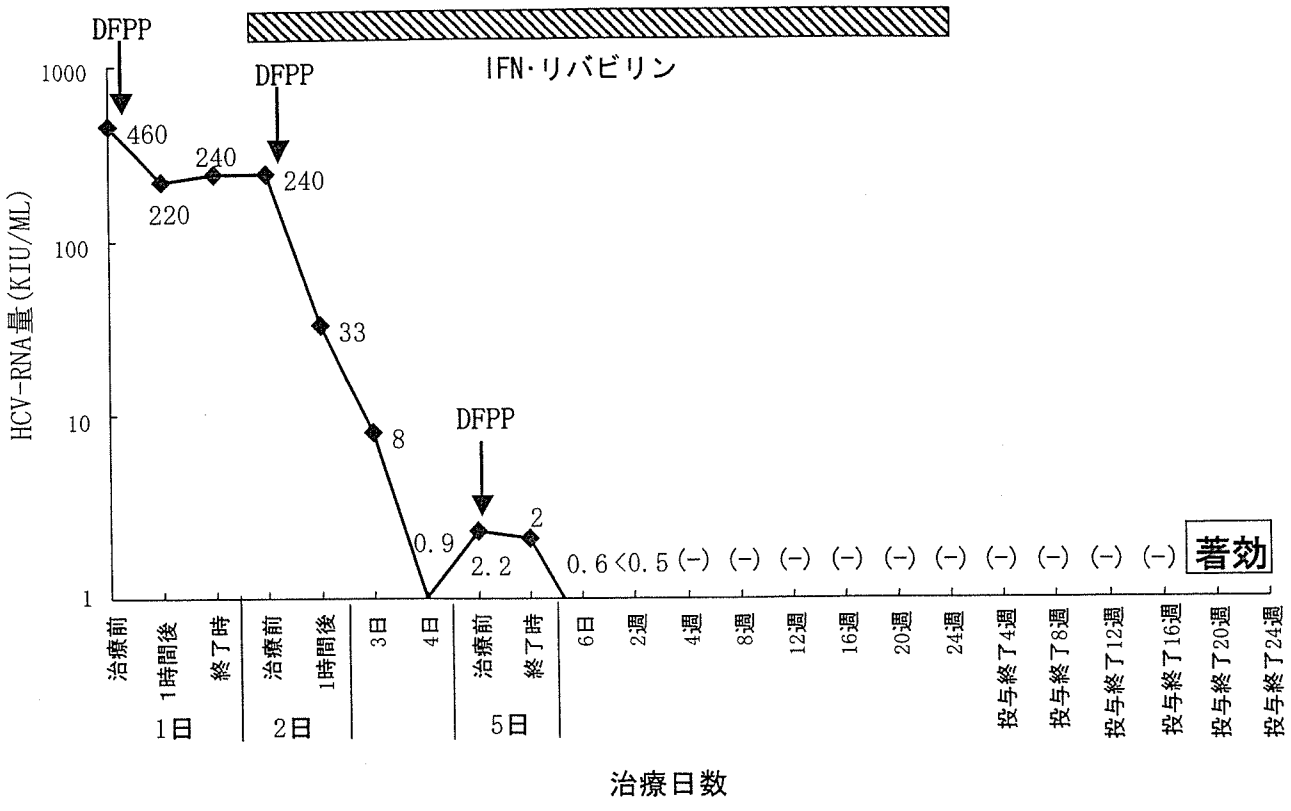

図2　IFN・リバビリンおよびDFPP併用例のHCV RNA量の動態（DFPP-1群，patient No. 1）
第1回目のDFPPでHCV　RNA量の減少が認められ，さらにIFN・リバビリン中の2回目のDFPP以降，著明なHCV RNA量の減少が認められている．この症例はIFN終了後もHCV RNA陰性が持続し著効となった．

終了時点では240 KIU/mlまで低下した．DFPP終了後1時間以内にIFN・Ribを投与した．翌朝，DFPP施行前のHCV RNA量は240 KIU/mlであり増加は認められなかった．2回目（2日目）のDFPP施行終了時点で33 KIU/mlまでHCV RNA量が低下した．DFPPは計3回施行したが，2週目でHCV RNA定量検査で検出限界（0.5 KIU/ml）以下となり，4週目のHCV RNA定性で陰性化を認め，最終的に著効となった．

3.3　DFPP施行による副作用

6症例，18回のDFPP施行による副作用では，気分不良が4件，嘔気が2件，血圧低下が1件認められた．発現時期は，DFPPの初回〜2回目までがほとんどであり，程度はいずれも軽度であり，生理食塩液を点滴静注することにより短時間で消失した．フィブリノーゲン値（mg/dl）は，治療前，1，2，3，4，5，6，7日目に測定し，203.6±41.16，122.0±20.6，96.6±24.1，108.0±27.6，112.0±28.9，132.2±43.9，195.6±35.5であった．DFPPの副作用やフィブリノーゲン低下により，IFN・Rib投与のスケジュールに支障をきたした症例はなかった．しかしフィブリノーゲン値が100 mg/dl以下になる症例も存在した．

4.　考　　察

Genotype 1 b，高ウイルス量例のいわゆる難治性のC型慢性肝炎に対しては，IFN・Rib療法が最も強力で，IFN製剤にペグを結合させたペグインターフェロン（PEG IFN）の48週間投与が現在の標準的治療法であるが，この治療法よりも強力な治療法はなく，次世代の抗ウイルス剤開発にはかなりの年月が必要と考えられている．（PEG）IFN・Rib療法の著効率を上げるには，投与期間の延長も有効と考えられるが，副作用も強く長期の投与は難しく，医療保険上も48週以上の使用は認められていない．よってさらなる治療法の改善が求められている．

一方，HCV粒子は，DFPPを使用することで，サイズセパレーションによりHCVを物理的に血中から除去することが可能であることがわかった．今回の検討でもDFPPによりHCVはほぼ完全に除去されていた．一方，我々の行ったDFPP以外にもLDL吸着法によりHCVを除去する方法も考えられるが，完全

表4　DFPPによる副作用

	症状	発現時期	程度	処置	経過	本治療用具との関係	因果関係判定の根拠	本治療中止の有無
1	気分不良	DFPP 1回目 25分後	軽度	生食 100 ml 点滴静注	30分後に消失	有り	体外循環中に起こったため	無し
2	気分不良 嘔気	DFPP 3回目 65分後	軽度	生食 100 ml 点滴静注	20分後に消失	有り	体外循環中に起こったため	無し
3	嘔気	DFPP 2回目 回収直前	軽度	処置無し (終了直前のため)	10分後に消失	有り	体外循環中に起こったため	無し
4	気分不良	DFPP 2回目 160分後	軽度	生食 400 ml 点滴静注	30分後に消失	有り	体外循環中に起こったため	無し
5	血圧低下	DFPP 2回目 240分後	軽度	生食 100 ml 点滴静注	30分後に消失	有り	体外循環中に起こったため	無し
6	気分不良	DFPP 1回目 105分後	軽度	生食 100 ml 点滴静注	20分後に消失	有り	体外循環中に起こったため	無し

には除去できないと考えられる．これまでLDLを除去することによりHCV RNA量が減少したとする報告はある[8]が，IFN治療と併用した報告はない．また体外循環によりIFN治療効果を高める試みも報告があるが，クリオグロブリン除去[9]や好中球除去[10,11]であり基本的なコンセプトが異なっている．

DFPPは我が国ではすでに全身性エリテマトーデスなど他の疾患では認可され日常臨床で使用されている．そこでHCVを除去しIFN治療効果を高める目的でDFPPを併用することが考案された．IFN治療開始後早期からHCVは減少していくため，治療開始早期にDFPPを併用するスケジュールを考案した．安全性なども考慮しIFN治療開始1週間以内に3回施行することとした．DFPP装置の二次膜前後での検討では，HCV除去が確実に行われていることが証明された．血中（体内）での検討でもDFPP施行前後でHCV RNA量は減少していた．さらに重要なことは治療開始後早期とくに8週，12週でのHCV RNA陰性化率が高く，最終的な著効率も高かったことである．IFN・Rib療法においては治療開始後のHCV RNA陰性化が早いと著効率が高いことがわかっており[1]，このことからもDFPPによりHCV RNA陰性化を早期にもたらすことは著効となる確率を上げ，有用であると考えられる．実際に今回のDFPP併用で最終的に対照群より高い著効率が認められ，DFPPの有用性が確認された．

DFPP併用により血中のHCVは除去されても，HCVは肝臓で主に増殖するので，有用性は低いという考えも存在する．しかし血液中のウイルスを減少させることそのものが免疫賦活作用ももたらす可能性があり[12]，単純なウイルス除去以外の変化がウイルス駆除に関与する免疫システムの増強を引き起こしているのかもしれない．さらに透析などの体外循環の際には生体内IFN活性が上昇するとの報告[13]もありDFPPでもIFNなどが誘導された可能性もある．

DFPPの副作用（表4）に関しては，気分不良，嘔気，血圧低下が起こったものの補液で改善し，全例でDFPPを3回施行することができた．フィブリノーゲン値は軽度低下したが，中止に到った症例はなかった．ただやはり透析室にて体外循環を行うことに抵抗を感じる患者も存在し，安全性も含め十分なインフォームドコンセントが必要と考えられた．

5. 結　　語

DFPPとIFN・Ribの併用療法は難治例のC型慢性肝炎に対し高い治療効果を有する可能性があり，さらに症例を増やし検討する必要があると考えられた．

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Influence of Genotypes and Precore Mutations on Fulminant or Chronic Outcome of Acute Hepatitis B Virus Infection

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The outcome of acute hepatitis B virus (HBV) infection is variable, influenced by host and viral factors. From 1982 through 2004, 301 patients with acute HBV infection entered a multi-center cross-sectional study in Japan. Patients with fulminant hepatitis (n = 40) were older (44.7 ± 16.3 vs. 36.0 ± 14.3 years, $P < .0017$), less predominantly male (43% vs. 71%, $P = .0005$), less positive for hepatitis B e antigen (HBeAg) (23% vs. 60%, $P < .0001$), less infected with subgenotype Ae (0% vs. 13%, $P < .05$), and more frequently with Bj (30% vs. 4%, $P < .0001$) than those with acute self-limited hepatitis (n = 261). Precore (G1896A) and core-promoter (A1762T/G1764A) mutations were more frequent in patients with fulminant than acute self-limited hepatitis (53% vs. 9% and 50% vs. 17%, $P < .0001$ for both). HBV infection persisted in only three (1%) patients, and they represented 2 of the 23 infected with Ae and 1 of the 187 with the other subgenotypes (9% vs. 0.5%, $P = .032$); none of them received antiviral therapy. In multivariate analysis, age 34 years or older, Bj, HBeAg-negative, total bilirubin 10.0 mg/dL or greater, and G1896A mutation were independently associated with the fulminant outcome. In *in vitro* transfection experiments, the replication of Bj clone was markedly enhanced by introducing either G1896A or A1762T/G1764A mutation. In conclusion, persistence of HBV was rare (1%) and associated with Ae, whereas fulminant hepatitis was frequent (13%) and associated with Bj and lack of HBeAg as well as high replication due to precore mutation in patients with acute HBV infection. *Supplementary material for this article can be found on the HEPATOLOGY website (http://interscience.wiley.com/jpages/0270-9139/suppmat/index.html).* (HEPATOLOGY 2006; 44:326-334.)

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBc, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; EIA, enzyme immunoassay; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; ALT, alanine aminotransferase.

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Received February 8, 2006; accepted April 27, 2006.

Supported in part by a grant-in-aid from the Ministry of Health, Labour and Welfare of Japan (H16-kaken-3), Uehara Memorial Foundation, Toyoaki Foundation, and Miyakawa Memorial Research Foundation.

The nucleotide sequences of HBV DNA isolates used in this study have been deposited in the international DNA database under accession numbers AB249373-AB249636.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.21249

Potential conflict of interest: Nothing to report.

Approximately 3 billion people, one half of the world population, have been exposed to hepatitis B virus (HBV), of whom approximately 350 million are persistently infected with it.[1] Acute infection with HBV resolves in the great majority but can induce fulminant hepatitis or go on to become chronic. Host and viral factors may influence fulminant or chronic outcome of acute HBV infection, but they are not fully defined.

Eight genotypes have been detected by a sequence divergence greater than 8% in the entire HBV genome of approximately 3,200 nucleotides (nt), and designated by capital alphabet letters from A (HBV/A) to H in the order of documentation.[2-5] They have distinct geographical distributions associated with severity of liver disease as well as response to antiviral therapies.[6-8] Furthermore, subgenotypes have been reported for HBV/A, B, and C and named Aa/A1 (Asian/African type) and Ae/A2 (European type),[9] Bj/B1 (Japanese type) and Ba/B2 (Asian type),[10] as well as Cs/C1 (Southeast Asian type) and Ce/C2 (East Asian type).[11-13] Increasing lines of evidence indicate that subgenotypes of HBV/A and B influence the replication of HBV and bear clinical relevance.[14-16] Furthermore, genotypes affect mutations in precore region and core promoter, thereby influencing the expression of hepatitis B e antigen (HBeAg).[8,17]

During the 23 years from 1982 to 2004, a multi-center cross-sectional study was conducted throughout Japan on 301 patients with acute hepatitis B. We examined the influence of genotypes/subgenotypes on their fulminant or chronic outcome. Furthermore, the influence of G1896A or A1762T/G1764A on replication of HBV was evaluated in an *in vitro* replication model.

Patients and Methods

Patients With Acute Hepatitis B. During 1982 through 2004, 336 consecutive cases of acute hepatitis B were registered in 16 hospitals throughout Japan. These hospitals were from the following eight areas: Hokkaido (represented by J.-H. K. and S.H.), Tohoku (T.K. and K.S.), Kanto (H.T., Y.A. and K.I.), Koshin (E.T. and S.O), Tokai (A.O., Y.T., E.O., M.S., R.U., M.M., and S.K.), Kinki (T.O.), Honshu/Shikoku (Y.M., K.H., and M.O.), and Kyushu (H.Y. and H.S.). The diagnosis of acute hepatitis B was contingent on a sudden onset of clinical symptoms of hepatitis and detection of high-titered antibody to hepatitis B core antigen (anti-HBc) of IgM class in serum. Patients with initial high-titered anti-HBc (≥90% inhibition by a 1:200 diluted serum) were excluded; they were diagnosed as exacerbation of chronic hepatitis B. Patients with acute hepatitis A, hepatitis C, or human immunodeficiency virus co-infection, and drug- or alcohol-induced acute hepatitis also were excluded; hepatitis D virus infection was not examined because of its extreme rarity in Japan.[18] Most of them were followed for clinical outcomes until the disappearance of hepatitis B surface antigen (HBsAg) during 24 weeks or longer after the presentation. The criteria of fulminant hepatitis are based on the report by Trey et al.,[19] with a slight modification in 1981 (Inuyama symposium, Aichi, Japan): coma of grade II or higher and prothrombin time less than 40% developing within 8 weeks after the onset. Serum samples were collected at the presentation and had been stored at −80°C. HBV genotypes, HBV DNA, and HBeAg were determined, and clinical outcomes of acute hepatitis were analyzed. The study protocol conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics Committees of the institutions. Every patient gave an informed consent for this study.

Serological Markers of HBV Infection. HBsAg was determined by hemagglutination (MyCell; Institute of Immunology Co., Ltd., Tokyo, Japan) or enzyme immunoassay (EIA) (Axsym; Abbott Japan, Tokyo, Japan), and HBeAg by enzyme-linked immunosorbent assay (F-HBe; Kokusai Diagnostic, Kobe, Japan) or chemiluminescent EIA (Fujirebio Inc., Tokyo, Japan). Anti-HBc of IgM and IgG classes were determined by radioimmunoassay (Abbott Japan).

Genotypes and Subgenotypes of HBV. The six major HBV genotypes (A-F) were determined serologically by EIA using commercial kits (HBV GENOTYPE EIA; Institute of Immunology). The method depends on the combination of epitopes on preS2-region products detected by monoclonal antibodies, which is specific for each of them.[20] HBV/G was determined by a slight modification of the polymerase chain reaction (PCR) with specific primers.[21]

Subgenotypes of HBV/A designated Ae prevalent in Europe and Aa frequent in Africa as well as Asia,9 which corresponds to subgroup A′ originally reported by Bowyer et al.,[22] were determined by PCR restriction fragment length polymorphism (RFLP) involving nucleotide conversions in an immediate upstream of the precore region that are specific for each of them.[16,23] HBV/Bj (Japanese type) lacking the recombination with C over the precore region and the core gene and Ba (Asian type) with the recombination were determined by its absence or presence on HBV DNA sequences, as well as RFLP based on specific nucleotide substitutions, after the methods described previously.[15,24]

Subgenotypes of HBV/C, Cs (Southeast Asian type) found only in Southeast Asia, including Vietnam, Myanmar, Thailand, Laos, Bangladesh, Hong Kong, and Southern China, and Ce (East Asian type), found in Far