

Fig 2. α -Fetoprotein (AFP) changes with IFN and SNMC administration Paired *t*-test was used. **P* < 0.05 was regarded as significant. NS, not significant.

level. In contrast, IFN is considered to down-regulate cell cycle progression at the transcriptional level and induce apoptosis via the IFN receptor-mediated JAK-STAT signaling pathway (17). This competing action of IFN against HCV-related protein may be a direct anticancer mechanism that inhibits HCC. Actually, a clinical study has demonstrated anticancer effects of IFN administration against intrahepatic recurrence after resection of HCC (18), and IFN has also been used to treat HCC in combination with anticancer agents such as 5-fluorouracil (19).

Many reports have cited elevated AFP baselines as an independent HCC risk factor (8, 9) along with age, gender, liver histology stage, and ethnicity in HCV-infected patients. In the present study, the AFP baseline was decreased in all IFN-treated patients, even IFN nonresponders. This indicates that IFN therapy, rather than liver-protective therapy, universally reduces the risk factors of HCC in HCC high-risk subjects with high AFP values and advanced liver disease. Therefore, therapeutic strategies, such as long-term administration of low-dose IFN, may inhibit HCC in patients who have failed to respond to routine IFN treatment. Further investigation is needed to evaluate IFN effect in relation to AFP production and hepatocarcinogenesis.

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HEPATOLOGY

Clinical features of hepatocellular carcinoma that occur after sustained virological response to interferon for chronic hepatitis C

MASAFUMI IKEDA,*[†] SHIGETOSHI FUJIYAMA,[‡] MOTOHIKO TANAKA,[‡] MICHIO SATA,[§] TATSUYA IDE,[§] HIROSHI YATSUHASHI[¶] AND HIROSHI WATANABE^{**}

*Department of Gastroenterology and Hepatology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, [†]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, [‡]Department of Gastroenterology and Hepatology, NTT West Kyushu General Hospital, Kumamoto, [§]Second Department of Internal Medicine, Kurume University School of Medicine, Fukuoka, [¶]Institute for Clinical Research, National Nagasaki Medical Center, Nagasaki and ^{**}Third Department of Internal Medicine, Fukuoka University School of Medicine, Fukuoka, Japan

Abstract

Background and Aim: This study investigated the clinical features of hepatocellular carcinoma in patients with sustained virological response to interferon for hepatitis C viral (HCV) infection.

Methods: A total of 7715 patients with HCV infection were treated with interferon and followed up for more than 1 year after withdrawal of interferon in 64 Japanese hospitals and clinics between July 1988 and August 2001. Sustained virological response was obtained in 2515 (32.6%) patients. Of these 2515 patients, clinical data were collected for 38 patients in whom hepatocellular carcinoma developed. Sustained virological response was defined as HCV RNA negativity more than 6 months after the termination of interferon.

Results: All patients were HCV RNA negative at the time of diagnosis of hepatocellular carcinoma. The median period until the detection of hepatocellular carcinoma was 4.7 years (range 1.4–9.0 years). There were significant improvements in hepatic function including serum albumin, aspartate aminotransferase, alanine aminotransferase, indocyanine green test, platelet count and histological activity grade in comparison with those before interferon therapy and at the onset of hepatocellular carcinoma. 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) ($P = 0.002$).

Conclusions: The present findings emphasize the importance of regular medical follow up of patients with HCV infection, as even patients showing a sustained virological response to interferon and in whom hepatic function has improved have the potential to develop hepatocellular carcinoma.

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Key words: follow-up, hepatitis C virus, hepatocellular carcinoma, interferon, sustained virological response.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of most prevalent malignant tumors worldwide, and its incidence is increasing. Most cases are attributable to chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection.^{1,2} In Japan, epidemiological studies have shown that HCV is more common than HBV as the causative

agent of HCC.^{3,4} Because HCV infection is related to the development of cirrhosis and HCC, it was assumed that eradication of this infection would provide the most effective means of preventing HCV-related complications, including HCC.

Currently, interferon (IFN), peginterferon or combination therapy with ribavirin, are the available drugs that are effective for terminating HCV infection. IFN

Correspondence: Dr Masafumi Ikeda, Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Email: masikeda@ncc.go.jp
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can induce a long-term favorable response and eradication of HCV RNA from serum after treatment cessation, although the response rate is not fully satisfactory.⁵⁻⁸ Furthermore, patients with HCV appear to derive a definitive benefit in terms of prevention of progression to cirrhosis and the development of HCC.⁷⁻¹⁵ However, even in some patients from whom HCV infection has been eliminated by IFN therapy, HCC can still be detected.¹¹⁻²⁷ In these patients, the clinical features of developing HCC have not been fully investigated,²⁰ although they have been documented in individuals or small numbers of cases.²¹⁻²⁷

The present study therefore attempted to elucidate the clinical features of HCC, especially the serial changes occurring in the period from before IFN therapy to the detection of HCC. A multicenter, collective study was conducted in the setting of hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, in Japan, as it was felt that a study conducted in a single institution would provide inadequate numbers of sustained responders who developed HCC.

METHODS

Patients

This study was conducted at major hospitals and clinics belonging to the Japanese Society of Gastroenterology, Kyushu Division, Japan. A patient cohort in whom HCC had been detected among sustained responders to IFN therapy for chronic hepatitis C was collected by means of data collection instruments. All of the patients included had tested positive for HCV RNA before IFN therapy, and were followed up after withdrawal of IFN therapy for more than 1 year prior to the end of August 2001. Sustained virological response (SVR) was defined as HCV RNA negativity for more than 6 months after termination of IFN therapy. Diagnosis of HCC was based either on histological examination or on typical computed tomographic and/or angiographic findings at each institution. Patients were excluded if HCC was detected within 1 year after the termination of IFN therapy, because in such cases it was highly likely

that the cancer had been present at the end of IFN treatment.

In Japan at the time of the study, the standard schedule was 6–10 MU IFN- α every day for the first 2–4 weeks and then three times a week for the following 20–22 weeks, or 6 MU IFN- β every day for 6–8 weeks. Patients treated with peginterferon or combination therapy with ribavirin were not included because these therapies had not been approved by the Ministry of Health, Labor and Welfare in Japan at the time of the study.

At the first data collection, hospitals were approached and information on the number of patients who had undergone IFN therapy for chronic hepatitis C and who had been followed up for more than 1 year after the termination of IFN therapy, the number of SVR patients among them, and the number of patients in whom HCC had developed among the SVR patients was requested; 64 hospitals responded, listed in Appendix I.

In the second data collection, carried out on SVR patients in whom HCC had developed, clinical data were requested for each patient from before IFN therapy and at detection of HCC.

Data collected

To elucidate the clinical features of HCC that developed in SVR patients, host-related, treatment-related and tumor-related variables before IFN therapy and at detection of HCC were investigated (Table 1). Assessments of the staging of liver fibrosis and the grade of inflammatory activity were based on the classification of Desmet *et al.*,²⁸ where staging is defined as: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis); and grading is defined as: A0 (no activity), A1 (mild activity), A2 (moderate activity), and A3 (severe activity).

Follow-up ended with the last recorded visit before 31 August 2001. The period until the detection of HCC was measured from the day of termination of IFN therapy to the day when HCC was first diagnosed by imaging modalities such as ultrasonography or computed tomography. The medical follow-up period for the detection of HCC after SVR was defined as the interval

Table 1 Clinical features of 38 patients with chronic hepatitis C in whom hepatocellular carcinoma (HCC) developed after sustained response to interferon

Clinical feature	Before interferon	At detection of HCC	P-value
Host-related variables			
Age (years) [median (range)]	60 (36–71)	64 (38–77)	<0.0001
<60 [n (%)]	16 (42%)	4 (11%)	
>60 [n (%)]	22 (58%)	34 (89%)	
Sex [n (%)]			
Men	34 (89%)	—	—
Alcohol abuse [n (%)] [†]			
Positive	2 (5%)	—	—
Viral load before interferon (copies/mL) [n (%)]			
>10 ⁶	2 (13%)	—	—

Table 1 Continued

Clinical feature	Before interferon	At detection of HCC	P-value
Serological group before interferon [<i>n</i> (%)]			
Group 1	6 (33%)	—	—
Group 2	12 (67%)	—	—
Hepatic function [median (range)]			
Platelet ($\times 10^4/\text{mm}^3$)	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 [<i>n</i> (%)]			
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 [<i>n</i> (%)]			
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 [<i>n</i> (%)]			
α	36 (95%)	—	—
β	2 (5%)	—	—
Total amount of interferon [median (range)]	480 (126–846)	—	—
Prior interferon therapy [<i>n</i> (%)]			
Positive	2 (5%)	—	—
Tumor-related variables			
Number of tumors [<i>n</i> (%)]			
Solitary	—	31 (82%)	—
Multiple (range)	—	7 (18%)	—
Maximum tumor size (mm)			
Median		30 (12–150)	
≤30 [<i>n</i> (%)]	—	21 (57%)	—
>30 [<i>n</i> (%)]	—	16 (43%)	—
Alpha-fetoprotein (ng/mL) [<i>n</i> (%)]			
>20	4 (16%)	15 (41%)	0.07
PIVKA-II (AU/mL) [<i>n</i> (%)]			
>0.063	0 (0%)	13 (43%)	0.01
Differentiation of HCC [<i>n</i> (%)]			
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 [‡] [<i>n</i> (%)]			
Resection	—	16 (43%)	—
Local ablation	—	10 (27%)	—
Transarterial treatment	—	11 (30%)	—

PIVKA-II, protein induced by vitamin K absence or antagonist-II; R₁₅, 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 R₁₅). 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

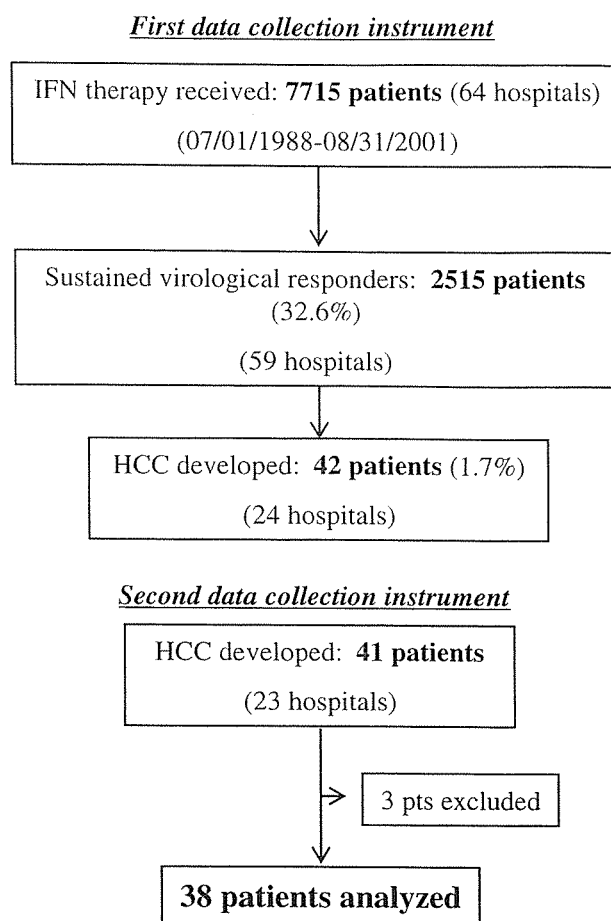


Figure 1 Profile of patients and data collection. One hospital did not respond to second data collection request. IFN, interferon; HCC, hepatocellular carcinoma.

25 mm; range 12–51 mm) (*P* = 0.002). Of the 38 patients, 16 underwent hepatic resection for HCC.

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

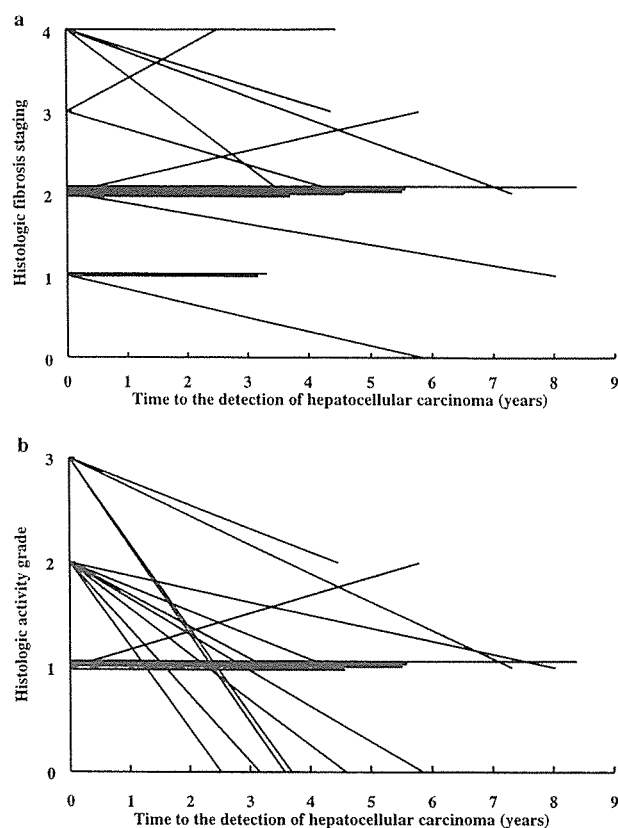


Figure 2 Serial changes in (a) histological fibrosis staging and (b) histological activity grade for each patient when compared before interferon therapy and at detection of hepatocellular carcinoma.

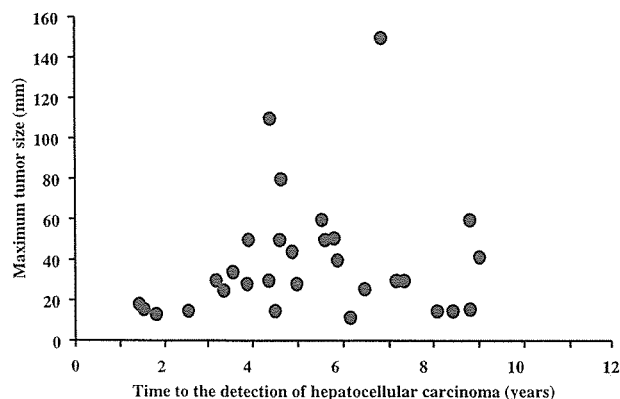


Figure 3 Maximum tumor size and time until detection of hepatocellular carcinoma.

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 HCC²⁰ 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 SVR²⁴ 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.

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- 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; Nishinohon 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; Hakujuji Hospital; Hakuaiikai Hospital.

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

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

KAZUO TANAKA¹, YUMIKO NAGAO^{1,2}, TATSUYA IDE^{1,2}, RYUKICHI KUMASHIRO¹ and MICHIO SATA^{1,2}

¹Second Department of Medicine and ²Department of Digestive Disease Information and Research, Kurume University School of Medicine, Asahi-machi, Kurume, Fukuoka 830-0011, Japan

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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 hyperendemic 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.

Correspondence to: Dr Kazuo Tanaka, Second Department of Internal Medicine, Kurume University School of Medicine, 67 Asahimachi, Kurume 830-0011, Japan
E-mail: wada.isi@alto.ocn.ne.jp

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

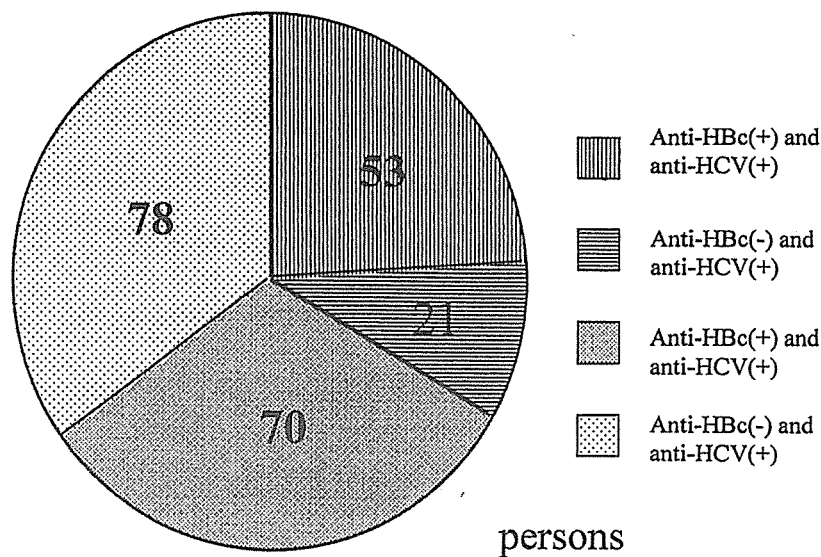


Figure 1. Diagram showing incident of hepatitis virus markers (anti-HCV and anti-HBc) among the 222 inhabitants 12 years ago. Fifty-three inhabitants were anti-HBc positive and anti-HCV positive, 21 were anti-HBc negative and anti-HCV positive, 70 were anti-HBc positive and anti-HCV negative, and 78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

^aP<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

^aP<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.

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C型慢性肝炎へのIFN α -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

井出 達也*¹・西田 秀美*²・矢野 淳子*²・田中 英明*²
奥田 誠也*²・佐田 通夫*¹

*¹ 久留米大学医学部内科学講座消化器内科部門, *² 同腎臓内科部門

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

Tatsuya Ide*¹, Hidemi Nishida*², Jyunko Yano*², Hideharu Tanaka*², Seiya Okuda*² and Michio Sata*¹

*¹ Division of Gastroenterology, and *² Division of Nephrology, Department of Medicine, Kurume University School of Medicine

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 1 b, 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 1 b, 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¹, 著効率は、20%前後であった²。最近で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³も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

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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F4, A3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IFN α -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
肝組織 (Grading)	0/1/2/3	0/1/2/0	0/2/9/0
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 / μ 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-08 W を、血漿成分分離器として EC-50 W 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、 $1,113.3 \pm 840.8$ 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、 $995.0 \pm 1,089.3$ 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												IFN 24週単独投与						投与終了後観察期間						判定										
			2W	4W	8W	12W	16W	20W	24W	28W	32W	36W	40W	44W	48W	4W	8W	12W	16W	20W	24W																
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	NT	(-)	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.

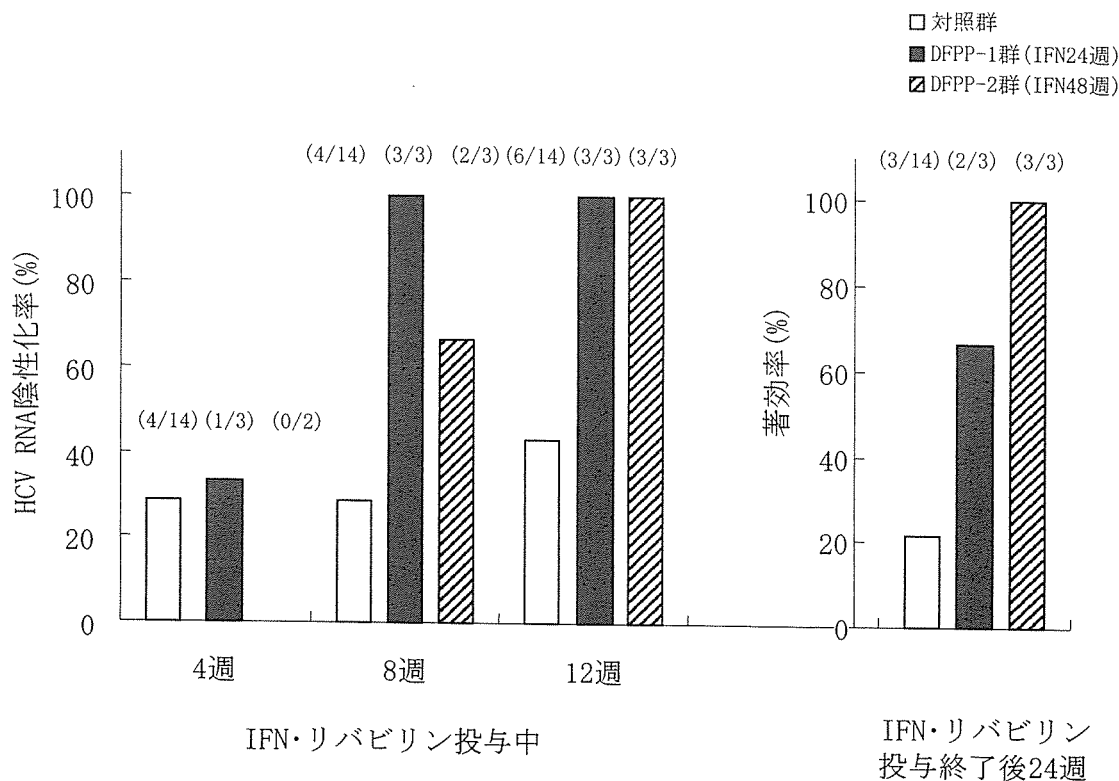


図1 HCV-RNA 陰性化率の推移と著効率

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0.7KIU/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460KIU/mlであったが, 1回目のDFPPの

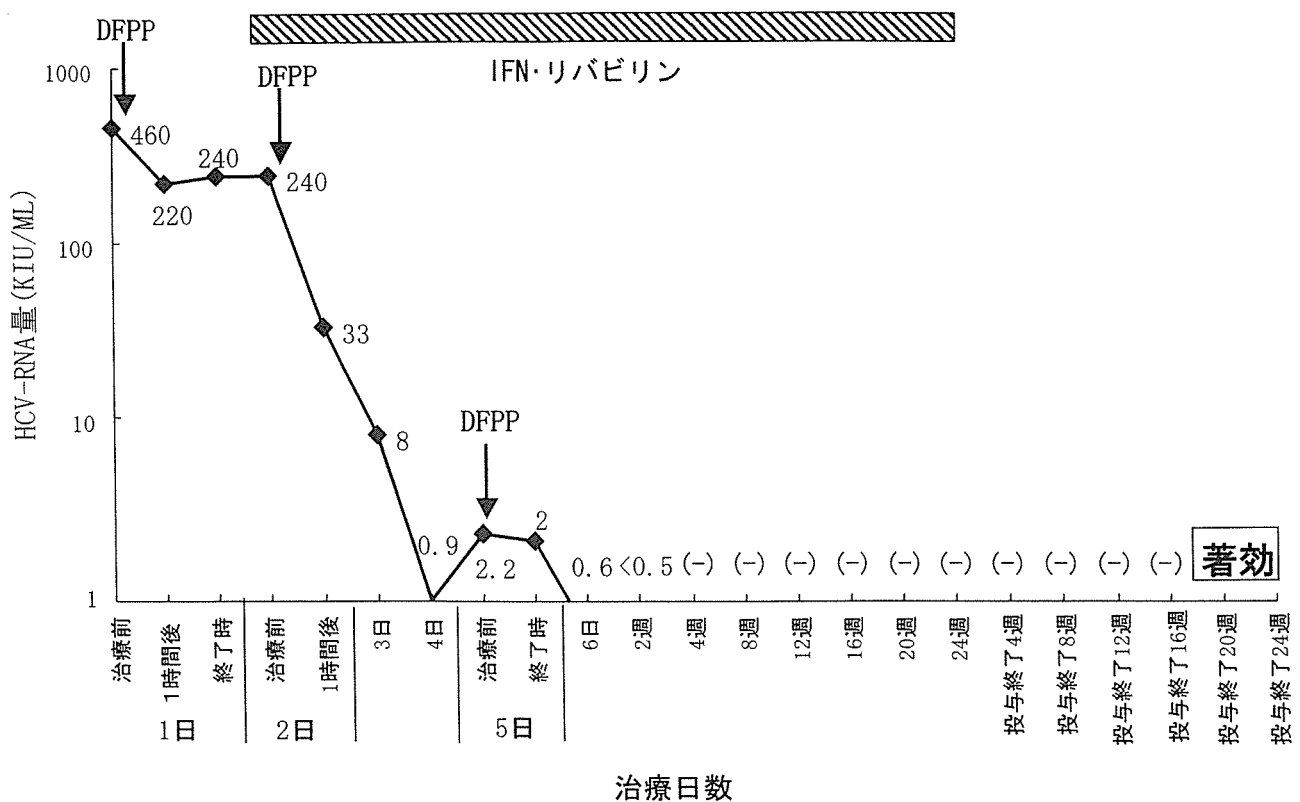


図2 IFN・リバビリンおよびDFPP併用例のHCV RNA量の動態 (DFPP-1群, patient No. 1)

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 1b, 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療法が最も強力であり、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