

Fig. 1 Study design

Bezafibrate has been used as an anti-hyperlipidemic agent because of its ability to lower cholesterol and triglyceride levels and elevate HDL-cholesterol²⁾. There have been several reports that bezafibrate improves liver function test data in patients with fatty liver³⁾. Recently, it was reported that bezafibrate was effective in the treatment of primary biliary cirrhosis (PBC)^{4~6)} and chronic hepatitis C^{7, 8)}. Ursodeoxycholic acid (UDCA) has been reported to improve liver function in patients with chronic viral hepatitis. This drug has been widely used in the treatment of chronic viral hepatitis, however, the effectiveness of this drug is limited⁹⁾. We evaluate the effectiveness of additional bezafibrate treatment in patients who had not responded to treatment with UDCA.

METHODS

1 Patients

The subjects were 50 patients with chronic hepatitis C who visited our hospital between April 2004 and March 2005. The diagnosis of chronic hepatitis was based on histological examination of liver specimens obtained by needle biopsy performed under ultrasonic guidance. The inclusion criteria were as follows : (1) having received no prior IFN therapy or having received the last dose of IFN 6 months or more prior to the start of this study ; (2) alanine aminotransferase (ALT) level over 60 IU/L after 12-week or longer treatment with UDCA (300 or 600 mg/day) prior to the start of this study ; (3) serum total cholesterol level below 260 mg/dL and triglyceride level below 300 mg/dL before the start of the study ; and (4) not complicated by HCC. If the results of abdominal ultrasonography or abdominal computed tomography suggested the presence of hepatocellular carcinoma, angiography or needle biopsy was performed under ultrasonic guidance. This study conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the Osaka City University Medical School. Written informed consent was obtained from all subjects (consent was given freely and voluntarily by each patient). Randomization was performed by placing the treatment assignments in sealed envelopes.

2 Study design

Two treatment regimens (UDCA treatment alone and combined UDCA and bezafibrate treatment) were used. The two regimens were applied following a cross-over study design (Fig. 1). In group A, UDCA treatment alone was continued for 16 weeks (300-600 mg/day), followed by 16 weeks of treatment with a combination of UDCA (300 or 600 mg/day) and bezafibrate (400 mg/day). In group B, 16 weeks of treatment with a combination of UDCA (300 or 600 mg/day) and bezafibrate (400 mg/day) was followed by 16 weeks of UDCA treatment alone (300 or 600 mg/day). Bezafibrate (Bezato SR) was purchased from Kissei Pharmaceutical Co., Ltd., Matsumoto, Japan. In each patient, the dose level of UDCA remained unchanged throughout the study.

During the study period, the following tests were conducted every 8 weeks : liver function tests (asparate

Table 1 Baseline characteristics

	Group A (n=25)	Group B (n=23)	p-value
Male/female	8/17	11/12	0.26
Age (yr) (range)	60.5±9.50 (30-74)	59.5±10.3 (43-78)	0.73
Height (cm)	158.1±9.2	162.4±9.1	0.13
Weight (kg)	61.3±13.0	62.4±14.9	0.80
Body mass index	24.4±4.3	23.5±4.1	0.46

Table 2 Baseline characteristics

	Group A (n=25)	Group B (n=23)	p-value
AST (IU/L)	85.5±29.7	82.2±29.8	0.29
ALT (IU/L)	107.0±42.6	111.4±46.3	0.92
γ-GTP (IU/L)	48.9±27.4	64.2±44.9	0.41
ALP (IU/L)	204.9±63.4	222.1±71.7	0.59
Cholinesterase (IU/L)	482.6±105.4	466.6±153.8	0.87
Total bile acid (μmol/L)	22.8±19.1	31.5±39.0	0.83
Total bilirubin (mg/dL)	0.8±0.3	0.9±0.2	0.87
Total cholesterol (mg/dL)	171.3±24.6	172.1±27.2	0.97
Triglyceride (mg/dL)	101.8±41.4	101.3±33.9	0.97
HDL-cholesterol (mg/dL)	57.9±12.4	56.7±12.5	0.80
Platelet counts (×10 ⁴ /μL)	15.0±4.1	14.9±5.2	0.80
HCV-RNA titer (IU/mL)	481.2±242.3	542.5±252.0	0.47

AST, aspartate aminotransferase ; ALT, alanine aminotransferase ;
γ-GTP, γ-glutamyl transpeptidase ; ALP, alkaline phosphatase

aminotransferase (AST), ALT, γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), cholinesterase, total bilirubin, total bile acid) ; serum lipids (total cholesterol, triglyceride, HDL-cholesterol) ; renal function tests (blood urea nitrogen (BUN), creatinine) ; common hematology tests (red blood cell count, white blood cell count, platelet count) ; and HCV-RNA titer.

3 Statistical analysis

Data were analyzed statistically using StatView Ver. 5 (SAS Institute Inc., NC, USA). Data related to the background of patients were tested using unpaired *t*-test or chi-square test. Each parameter was expressed as mean ± SD. The Wilcoxon signed rank test was employed for intra-group comparison and the Mann-Whitney test was used for inter-group comparison. Logistic regression analysis was used for analysis of factors used to determine drug effectiveness, and the odds ratio and 95% confidence interval were calculated. The significance of differences was determined using a two-tailed test. *p* < 0.05 was regarded statistically significant, and *p* < 0.1 as indicating a tendency for significant differences.

RESULTS

1 Patient characteristics

Forty-eight patients (25 in group A and 23 in group B) completed 32 weeks of observation. No adverse effects of bezafibrate were noted. None of the background variables differed significantly between groups A and B at the start of the study (**Table 1**). There was no significant difference between groups A and B in any hematological parameter or HCV-RNA titer at the start of the study (**Table 2**).

2 Changes in liver function tests

In both groups A and B, ALT levels during the combined bezafibrate and UDCA treatment period were significantly lower than levels before the start of the study. In group B, ALT levels during the bezafibrate combined treat-

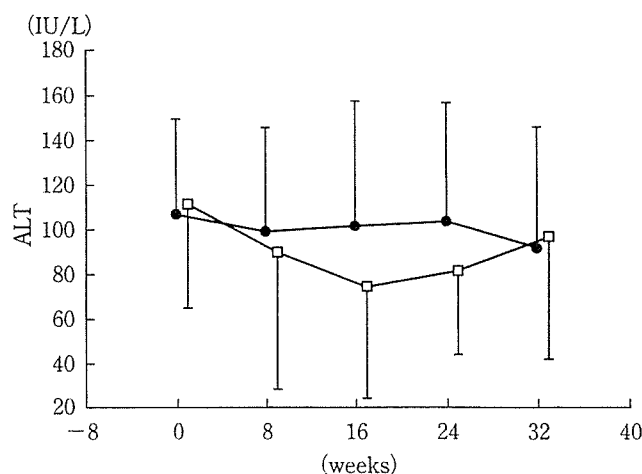


Fig. 2 Changes in ALT

In both groups A and B, ALT levels during the combined bezafibrate and UDCA treatment period were significantly lower than levels before the start of the study.

In group B, ALT levels during the bezafibrate combined treatment period were significantly lower than the levels recorded at the end of the UDCA treatment period.

●, Group A ; □, Group B

ment period was significantly lower than levels recorded at the end of the UDCA treatment period (Fig. 2). In both groups, γ -GTP and ALP levels during the bezafibrate combined treatment period were significantly lower than those before the start of the study. In group B, the levels of these two parameters during the bezafibrate combined treatment period were significantly lower than those at the end of the UDCA treatment period (Fig. 3, 4). A similar trend was also observed in AST and total bilirubin levels.

3 Changes in serum lipids

In both groups A and B, total cholesterol and triglyceride levels during the combined bezafibrate and UDCA treatment period were significantly lower than those before the start of the study. In group B, the levels of these two parameters during the bezafibrate combined treatment period were significantly lower than those at the end of the UDCA treatment period (Fig. 5, 6). HDL-cholesterol showed no significant change.

4 Changes in hematological tests and HCVRNA level

In both groups A and B, platelet counts during the combined bezafibrate and UDCA treatment period were significantly higher than those before the start of the study. In group A, platelet counts tended to decrease during the UDCA treatment period, but levels of this parameter during the bezafibrate combined treatment period were significantly higher than those recorded at the end of the UDCA treatment period. In group B, platelet counts during the bezafibrate combined treatment period were significantly higher than those at the end of the UDCA treatment period (Fig. 7). Red blood cell and white blood cell counts showed no significant change. Neither group A nor group B showed a significant change in HCV-RNA titer (Fig. 8).

5 Logistic regression analysis

Following treatment with bezafibrate, ALT decreased to the normal range (below 50IU/L) in 13 patients

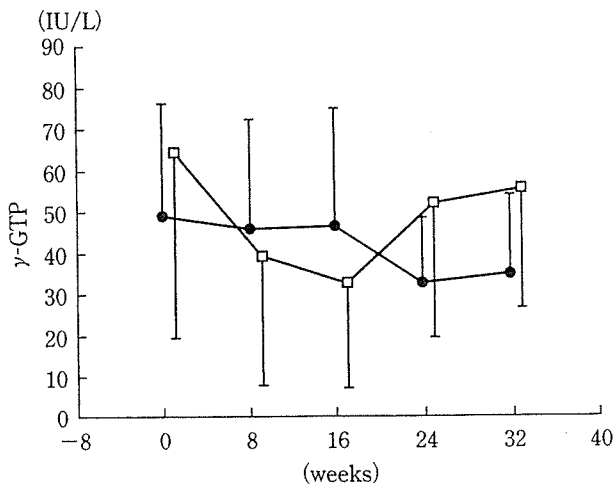


Fig. 3 Changes in γ -GTP

In both groups A and B, γ -GTP levels during the combined bezafibrate and UDCA treatment period were significantly lower than levels before the start of the study.

In group B, γ -GTP levels during the bezafibrate combined treatment period were significantly lower than levels recorded at the end of the UDCA treatment period.

●, Group A ; □, Group B

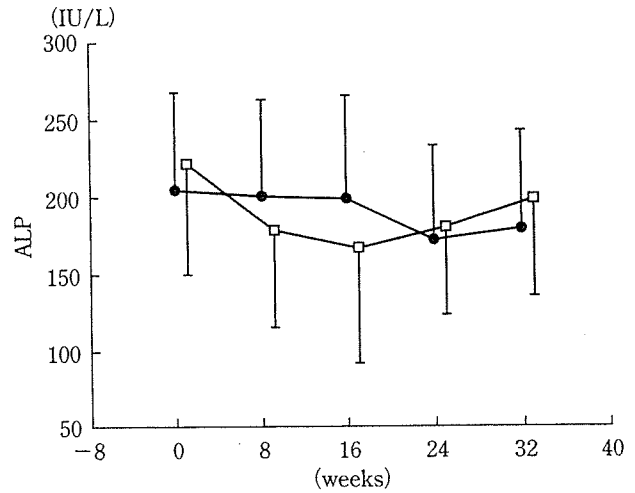


Fig. 4 Changes in ALP

In both groups A and B, ALP levels during the combined bezafibrate and UDCA treatment period were significantly lower than levels before the start of the study.

In group B, ALP levels during the bezafibrate combined treatment period were significantly lower than levels recorded at the end of the UDCA treatment period.

●, Group A ; □, Group B

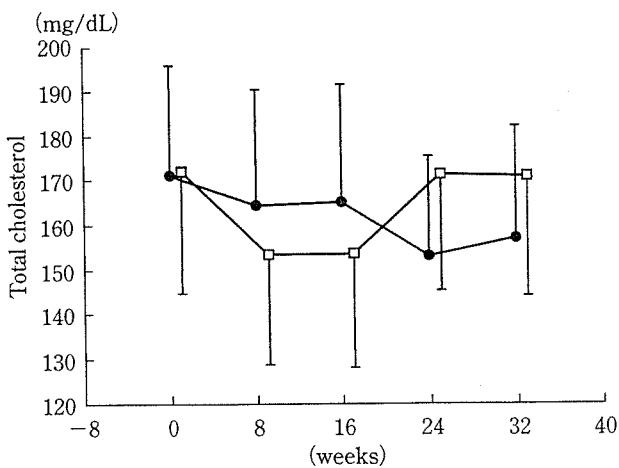


Fig. 5 Changes in total cholesterol

In both groups A and B, total cholesterol levels during the combined bezafibrate and UDCA treatment period were significantly lower than levels before the start of the study.

In group B, total cholesterol levels during the bezafibrate combined treatment period were significantly lower than levels recorded at the end of the UDCA treatment period.

●, Group A ; □, Group B

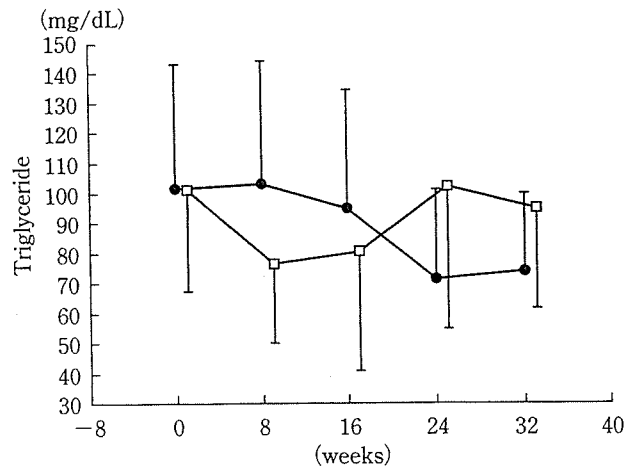


Fig. 6 Changes in triglyceride

In both groups A and B, triglyceride levels during the combined bezafibrate and UDCA treatment period were significantly lower than levels before the start of the study.

In group B, triglyceride levels during the bezafibrate combined treatment period were significantly lower than levels recorded at the end of the UDCA treatment period.

●, Group A ; □, Group B

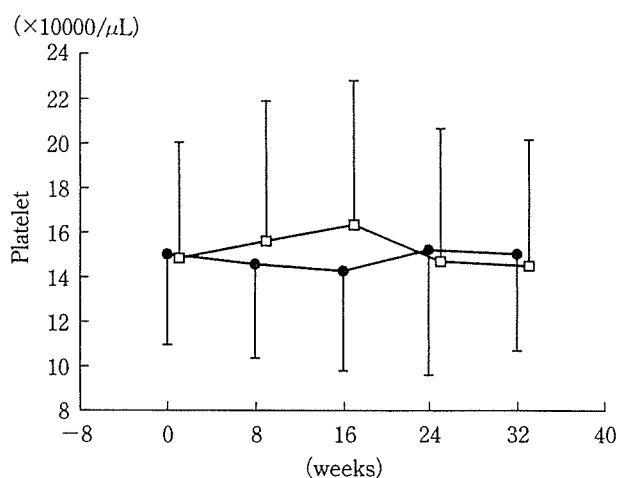


Fig. 7 Changes in platelet count

In both groups A and B, platelet counts during the combined bezafibrate and UDCA treatment period were significantly higher than the counts before the start of the study. In group B, platelet counts during the bezafibrate combined treatment period were significantly higher than the counts recorded at the end of the UDCA treatment period.

●, Group A ; □, Group B

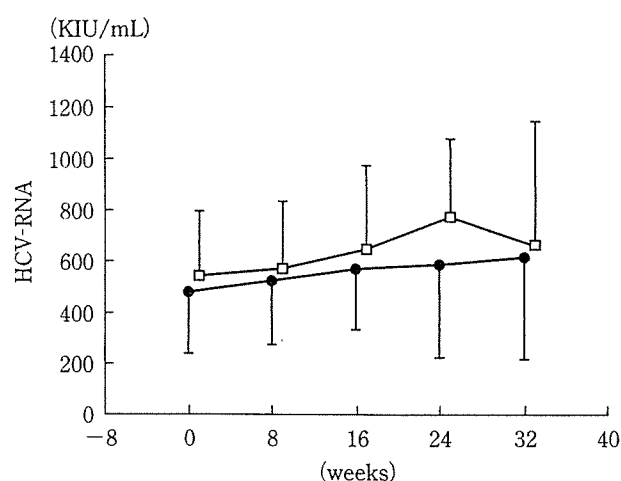


Fig. 8 Changes in HCV-RNA

Neither group A and B showed a significant change in HCV-RNA titer.

●, Group A ; □, Group B

Table 3 Logistic regression analysis

	Univariate			Multivariate		
	Odds ratio	(95% CI)	p-value	Odds ratio	(95% CI)	p-value
Age	1.08	(0.99-1.17)	0.089	1.07	(0.96-1.20)	0.24
ALT	0.97	(0.95-1.00)	0.065	0.98	(0.94-1.01)	0.17
BUN	1.27	(1.01-1.60)	0.039	1.13	(0.90-1.44)	0.29
Platelet count	1.14	(0.98-1.33)	0.087	1.20	(0.97-1.49)	0.096

ALT, alanine aminotransferase ; BUN, blood urea nitrogen

(responders) but did not decrease to the normal range in 32 patients (non-responders). Logistic regression analysis was conducted in these two patient groups (the responder group and the non-responder group) using background variables and pre-treatment test data. Univariate analysis revealed that BUN served as a significant factor and that age, ALT, and platelet count tended to serve as quasi-significant factors (i.e., factors responsible for significant differences in response to treatment). In multivariate analysis, no significant factor was identified, and platelet count served as a quasi-significant factor (Table 3).

DISCUSSION

Regarding the effectiveness of bezafibrate for liver disease, a number of reports have shown that treatment with this drug improves liver function test data in patients with fatty liver³⁾. The effectiveness of this drug in the treatment of PBC has also been reported by many investigators^{4~6)}. The Ministry of Health, Labour and Welfare

Study Group on Intractable Liver Disease, Japan, is conducting a prospective study on the efficacy of this drug in the treatment of PBC. It was recently reported that bezafibrate was effective in the treatment of chronic hepatitis C. Kurihara, et al⁷⁾. administered bezafibrate (400 mg/day) for 6 months to 30 patients with chronic hepatitis C and found a significant reduction in AST, ALT, γ -GTP, and HCV-RNA levels. Fujita, et al⁸⁾. administered bezafibrate (400 mg/day) for 8 weeks to 6 patients with chronic hepatitis C who had not responded to IFN therapy, and found a significant reduction in HCV-RNA titers. In the same study, AST and ALT also decreased but not significantly. In patients showing reduction of AST and ALT in the same study, liver biopsy showed reduced inflammation. However, none of these reports was based on a case-control study.

In the present study designed as a cross-over test, we evaluated the effectiveness of additional bezafibrate treatment in patients who had not responded to treatment with UDCA. Additional treatment with bezafibrate resulted in significant reduction in ALT, γ -GTP and ALP levels. However, HCV-RNA titer showed no significant change following this treatment. Multivariate analysis showed that indicators of liver function improved more markedly in patients with a higher pre-treatment platelet count. This result indicates that bezafibrate is more effective in patients whose liver disease is less advanced.

Several hypotheses have been proposed to explain the mechanism for the effect of bezafibrate in improving the liver function. Bezafibrate stimulates the expression of MDR3-P-glycoprotein and, as a result, phospholipid stimulates micelle formation from hydrophobic bile acid, leading to protection of the liver from the cytotoxicity of this acid^{10, 11)}. According to another view, bezafibrate is a ligand of peroxisome proliferator activated receptor (PPAR) α and β ¹²⁾. The physiological role of PPAR- β has not been completely elucidated, but it has been discovered that PPAR- α is involved in inhibition of the inflammatory response¹³⁾. Leukotriene B₄, an activator of white blood cells, plays a central role in this inhibition.

In patients with chronic hepatitis C, persistence of hepatitis can lead to liver cirrhosis and eventually to HCC. Although IFN has been widely used for the treatment of chronic hepatitis C, its efficacy is not satisfactory. Currently, the most effective treatment for chronic HCV infection is combined treatment with IFN and ribavirin, but sustained viral eradication is seen in only 28% of patients infected with genotype-1b¹⁴⁾. Furthermore, because of the high cost of IFN and various fatal side effects, it is often difficult to continue IFN therapy for prolonged periods of time. A new effective therapy as an adjunct to IFN therapy is now desired. UDCA is more economical and can be used safely in such cases. For these reasons, UDCA is frequently adopted in the treatment of chronic hepatitis C. However, patients with chronic hepatitis C sometimes show no response to this therapy. When dealing with such resistant cases, it is possible to improve response by using UDCA in combination with bezafibrate. In the present study, combined UDCA and bezafibrate therapy improved liver function tests such as ALT but failed to reduce HCV-RNA titer. It has been reported that even when HCV-RNA does not become negative in response to IFN therapy, the onset of HCC can still be reduced significantly if ALT can be controlled to below 80 IU/L. With the combined UDCA and bezafibrate therapy evaluated in this study, eradication of HCV cannot be expected, but it seems possible to suppress the progression of liver lesions and the onset of HCC by reducing ALT level. This therapy is promising as a means of improving the quality of life of patients with chronic hepatitis C.

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Timing of interferon therapy and sources of infection in patients with acute hepatitis C

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Abstract

Background/Aims: Controversy over the selection of patients and optimum therapeutic method for acute hepatitis C has continued. The aims of this study were to investigate the source of infection, and to evaluate the timing of interferon (IFN) therapy in patients with acute hepatitis C in Japan.

Methods: The records of 102 patients from 12 facilities in Japan who developed acute hepatitis C after 1990 were investigated. In the patients treated with IFN, we performed multivariate analysis to investigate factors related to sustained virological response (SVR).

Results: Medical procedure was the most common source of infection, accounting for 32.4% in the 102 patients (33/102). Of 81 patients treated with IFN, 71 patients were followed after IFN therapy, and 57/71 (80.3%) had SVR. The SVR rate was significantly higher in patients treated with IFN within 24 weeks from onset of symptoms than the SVR rate in those treated after 25 weeks ($P=0.0016$). Multivariate analysis revealed that only the duration between onset of symptoms and initiation of IFN therapy (within 24 weeks) was related to SVR.

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Abbreviations: HCV, hepatitis C virus; IFN, interferon; ALT, alanine aminotransferase; SVR, sustained virological response; Peg-IFN, pegylated interferon

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Conclusions: Our multicenter cooperative survey revealed that medical procedure was the most frequent source of infection in acute hepatitis C. As concerns the therapy, interferon treatment should be initiated within 24 weeks after onset of symptoms.
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Keywords: Hepatitis C virus (HCV); Acute hepatitis; Medical procedure; Interferon

1. Introduction

There are about 170 million people infected with the hepatitis C virus (HCV) worldwide [1], and the infection progresses to hepatic cirrhosis in 10–30% [1,2]. Since patients often lack subjective symptoms even in acute hepatitis C [3], infection is often realized by patients when the pathology progresses to hepatic cirrhosis and hepatocellular carcinoma. There are a variety of sources of infection, such as medical procedure, intravenous drug use, and sexual behavior [4,5]. In addition, vertical transmission of HCV has been reported, and it seems that maternal viral load is significant for infection to fetus [6]. On the other hand, as a therapy for acute hepatitis C, interferon (IFN) administration has been established to be effective [4,5,7–13].

Although the initial prevention of hepatitis C virus (HCV) infection is ideal, the most effective method of preventing progression to the chronic hepatitis C is still controversial in the acute phase. In Japan, the development of acute hepatitis C due to blood transfusion has markedly decreased after introduction of the HCV antibody test for screening of blood donors [14]. However, infection from intravenous (i.v.) drug use and incidences due to accidental contamination of medical staff are still important problems [15,16]. Investigation for the sources of infection in acute hepatitis C is very important for the prevention. In this study, we investigated a national survey on the route of infection of acute hepatitis C and the therapeutic effectiveness according to the timing of IFN therapy. This survey consists of the largest number of case reports and may reflect the current situation of acute hepatitis C in Japan.

2. Patients and methods

2.1. Patients

A retrospective study was performed in patients of 12 facilities nationwide who developed acute hepatitis C after 1990. The total number of patients at the facilities was 102. Informed written consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Age, gender, source of infection, HCV serotype or genotype, HCV-RNA level, histology of liver biopsy, fluctuation in alanine aminotransferase (ALT) level, presence or absence of IFN therapy, course when not treated with IFN, duration between onset of symptoms and IFN therapy, type of IFN, total dose of IFN, administra-

tion method, total duration of administration, and therapeutic results were investigated in each patient.

2.2. Diagnosis of acute hepatitis C

The diagnostic criteria of acute hepatitis C were HCV-RNA detectable at the time of an elevated ALT level, followed by development conversion of HCV antibody. Patients in whom HCV antibody was already positive at the onset were excluded.

2.3. Natural course

In patients who followed the natural course without any treatments, the chronic hepatitis was defined as persistence of HCV-RNA positivity for 6 months or longer, and resolution was defined as a disappearance of serum HCV-RNA within 6 months followed by persistent negativity for 6 months or longer.

2.4. Definition of fluctuation of ALT

In patients diagnosed with acute hepatitis C, when one peak of the serum ALT level was observed, the fluctuation was designated as monophasic, and when two or more peaks were observed, the fluctuation was designated as bi- or multiphasic.

2.5. Serologic tests

Anti-HCV antibody was determined using a second-generation or third-generation enzyme-linked immunosorbent assay (Ortho Diagnostics Systems, Tokyo, Japan). Hepatitis C virus RNA was quantified by using the bDNA signal amplification assay (Chiron Corp.) or the Cobas Amplicor HCV Monitor test ver1.0 or 2.0 (Roche Diagnostic Systems, Tokyo, Japan). The data were represented as Meq/ml, K copies/ml, and KIU/ml, respectively. Detection of HCV-RNA to determine the response of IFN treatment was used by Amplicor HCV (Roche Diagnostics K.K., Japan). Hepatitis C virus serotype was determined using the genotyping enzyme-linked immunosorbent assay (International Reagents Corporation, Tokyo, Japan) to be type 1 or 2 [17].

2.6. IFN therapy

For IFN, IFN- α (natural form, gene recombinant, or consensus IFN), or IFN- β was used (Table 4). No concurrent treatment with IFN and ribavirin was administered to any patient. Among patients treated with IFN, the sustained

virological response (SVR) was defined undetectable HCV-RNA in serum at least 6 months after cessation of therapy. Non-response was defined as detectable HCV-RNA for 6 months after cessation of therapy.

2.7. Statistical analysis

Data were expressed as the mean \pm standard deviation for continuous variables and as counts for categorical variables. The results were compared using the Chi-square test, Fisher's exact probability test, or Mann-Whitney *U*-test, depending upon the type of data analysed. Logistic regression was used to analyse the factors contributing to SVR with IFN therapy. *P* values <0.05 were considered significant. Statistical analyses were performed by using Stat View software (version 5.0; SAS Institute Inc., Cary, NC).

3. Results

3.1. Patient characteristics

The baseline characteristics of the 102 patients in this study are shown in Table 1. The distribution of patients by gender and age is shown in Table 2.

3.2. Natural course

The natural course of the disease was followed in 21 patients, and the course could be followed to the outcome

Table 2
Distribution of patients according to gender and age

Age (years)	Number of patients					
	Medical procedure (M/F)	Accidental needlestick (M/F)	Sexual behavior (M/F)	Drug abuse (M/F)	Tattoo (M/F)	Unknown (M/F)
<19	0/1	0/0	0/0	0/1	0/0	0/1
20–29	5/1	3/8	1/3	2/1	3/0	2/6
30–39	4/3	3/3	2/1	0/1	0/0	3/3
40–49	2/4	0/4	1/0	0/1	0/0	2/3
50–59	4/3	0/0	0/0	0/0	0/0	2/3
60–69	4/1	0/0	0/0	0/0	0/0	2/0
70–79	0/0	0/0	0/0	0/0	0/0	1/1
>80	0/1	0/0	0/0	0/0	0/0	0/2
Total	19/14	6/15	4/4	2/4	3/0	12/19

M, male, F, female.

Table 3
Base-line characteristics of 18 untreated patients

	Resolved group (seven cases)	Chronic group (11 cases)	<i>P</i> value
Age	64.4 \pm 15.2	45.6 \pm 14.3	0.0331 ^a
Gender (male/female)	2/5	4/7	>0.9999
HCV RNA level (high ^b /low/N.D.)	2/4/1	6/4/1	0.6084
Serogroup (1/2/N.D.)	4/0/3	4/2/5	0.4667
Fluctuation of ALT level (monophasic/bi- or multiphasic/N.D.)	5/0/2	0/8/3	0.0008 ^a

N.D., not determined; ALT, alanine aminotransferase. Fluctuation of ALT level: monophasic; one peak of the serum ALT was observed, bi- or multiphasic; two or more peaks of the serum ALT were observed (N.D. was excluded from statistical comparisons).

^a Statistically significant.

^b Viral load (high): more than 100 KIU/ml or 1 Meq/ml.

Table 1
Base-line characteristics of 102 patients

Age	38.6 \pm 16.2 (16–84)
Male/female (mean age)	46 (39.2 \pm 16.0)/56 (38.2 \pm 16.5)
Source of infection (%)	
Medical procedure	33 (32.4)
Accidental needle stick	21 (20.6)
Sexual behavior	8 (7.8)
Drug abuse	6 (5.9)
Tattoo	3 (2.9)
Unknown	31 (30.4)
Viral load (high ^a /low/N.D.)	46/45/11
HCV serotype(1/2/N.D.)	54/23/25
IFN/without IFN	81/21

N.D., not determined; IFN, interferon. Details of the routes in medical procedure: surgery 14, blood transfusion 5, endoscopy 3, intravenous injection 4, invasive procedure 3, dental therapy 3, dialysis 1.

^a Viral load (high): more than 100 KIU/ml or 1 Meq/ml.

in 18 patients (the prognosis was unknown in three patients) (Table 3). The disease progressed to chronic hepatitis C in 61.1% of the patients and resolved spontaneously in 38.9% of the patients. The age and the fluctuation pattern of the ALT level were significantly different between the two groups. As for gender, serum HCV-RNA level, and serogroup, no correlation with spontaneous resolution or chronic hepatitis C was observed.

3.3. IFN therapy

Table 4 shows the backgrounds of the 81 patients treated with IFN. Of 71 patients in whom the effect was clarified,

Table 4
Base-line characteristics of 81 patients treated with interferon

Age	38.6 ± 16.2
Gender (male/female)	43/38
HCV RNA level (high ^a /low/N.D.)	38/36/7
HCV serogroup (1/2/N.D.)	46/21/14
Fluctuation of ALT level (monophasic/bi- or multiphasic/N.D.)	21/53/7
Type of IFN (α/β)	63/18
Total IFN dose (MU)	470 ± 228.1 (52–972)
Duration of IFN administration (w)	17.6 ± 8.9 (4.0–42.0)
Outcome (SVR ^b /NR/N.D.)	57/14/10

N.D., not determined; ALT, alanine aminotransferase; IFN, interferon; MU, million units; SVR, sustained virological response; NR, non-response: detectable HCV RNA in serum for 6 months after cessation of therapy.

^a HCV RNA level (high): more than 100 KIU/ml or 1 Mcq/ml.

^b Sustained virological response: undetectable HCV RNA in serum at least 6 months after cessation of therapy.

57 patients (80.3%) had SVR. Table 5 shows the results of the logistic regression analysis of SVR-related factors. Age, gender, serogroup, HCV-RNA level, fluctuation of ALT, duration between onset and initiation of IFN, type of IFN, total IFN dose, and duration of IFN administration were evaluated statistically by univariate and multivariate analysis. On multivariate analysis as well as univariate analysis, the duration between onset of symptoms and initiation of IFN therapy was the only factor related to SVR.

The SVR rate according to the duration before initiation of IFN therapy was investigated (Fig. 1), and the SVR rate was found to be significantly higher in patients treated before 24 weeks than in patients treated after 25 weeks. However, immediate administration has not been associated with higher SVR rate (0–8 weeks versus 9–24 weeks).

On comparison of the SVR rate by the source of infection, the SVR rate was 100% in the patients infected by accidental needlestick (19/19) (the prognosis was unknown in two of 21 patients infected by needlestick). This was significantly higher than that in patients infected via other routes (19/19

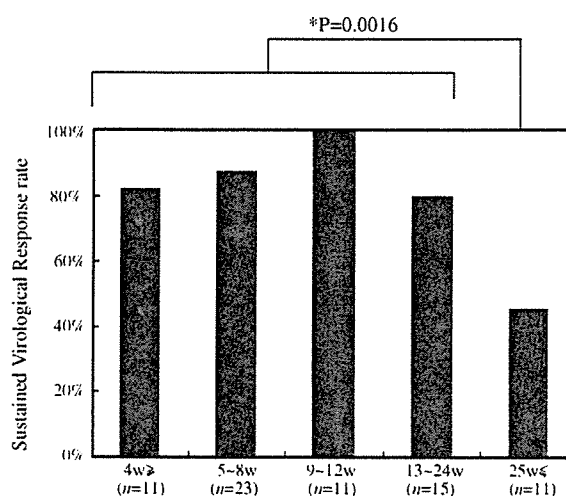


Fig. 1. Sustained virological response rate according to duration between onset of symptoms and initiation of IFN therapy. The groups treated with IFN 0–24 weeks after onset of symptoms and treated after 25 weeks were compared. Comparison by the Chi-square test. (*) Statistically significant; w, week.

versus 38/52, $P < 0.05$). The duration between onset of symptoms and initiation of IFN therapy was investigated according to the source of infection, and the duration was shortest in the needlestick group (9.7 ± 5.3 weeks).

4. Discussion

We examined the source of infection and optimal timing of therapy in patients with acute hepatitis C at 12 facilities in Japan. Since there has been no study performed in more than 100 patients with acute hepatitis C in Japan, this study may reflect the current situation in Japan. HCV serogroup of 25 patients were not determined (Table 1). Several reasons are considered. Firstly, the study is retrospective. Secondly,

Table 5
Logistic regression analysis of odds ratio for sustained virological response

Variable	Odds ratio	95% CI	P value
Univariate			
Age(40>/40≤)	2.48	0.73–8.46	0.147
Gender (female/male)	2.48	0.74–8.33	0.143
Serogroup (1/2)	1.03	0.23–4.54	0.969
HCV RNA level (high ^a /low)	1.75	0.46–6.68	0.413
Fluctuation of ALT (monophasic/bi-or multiphasic)	1.57	0.38–6.45	0.531
Duration between onset and initiation of IFN (≤24w/≥25w)	7.50	1.85–30.48	0.005 ^b
Type of IFN (alpha/beta)	4.33	0.52–36.18	0.176
Total IFN dose (>300MU/≤300MU)	2.27	0.63–8.15	0.208
Duration of IFN administration (≥24w/<24w)	1.43	0.44–4.67	0.551
Multivariate			
Duration between onset and initiation of IFN (≤24w/≥25w)	15.78	1.37–181.61	0.027 ^b

ALT, alanine aminotransferase; IFN, interferon; MU, million units; 95% CI, 95% confidence interval.

^a HCV RNA level high: More than 100 KIU/ml or 1 Mcq/ml.

^b Statistically significant.

titer of anti-HCV is often low in early phase of acute hepatitis C. Many patients were considered to be infected during a medical procedure. Studies on risk of surgery for the development of acute hepatitis C have been reported previously [18]. Alfonso et al. performed a large-scale surveillance in Italy and found that 25.5% of patients (261/1023) with acute hepatitis C had undergone an invasive procedure. Therefore, medical care should be recognized as an important source of infection in the sporadic incidence of acute hepatitis C. On the other hand, in blood donors of Western Mexico, the most frequent risk factors for HCV transmission were transfusion (42%) and household exposure (14.8%) [19]. Therefore, the main risk factors for infection may differ with countries.

Since IFN therapy for acute hepatitis C is not covered by the health care insurance, the therapy could not be administered to all patients. The progression to the chronic hepatitis C in the 18 patients with natural courses without IFN therapy was almost consistent with previous reports [20,21]. As shown in Table 3, a significant difference was observed in age, but this may have been due to the two patients in their 80s in the spontaneous resolution group (data not shown). The important point is that the ALT fluctuation was monophasic in all patients in the spontaneous resolution group. In contrast, the fluctuation was bi- or multiphasic in patients who progressed to chronic hepatitis C. As a characteristic of acute hepatitis C in which spontaneous elimination of the virus is likely to occur, it has been reported that many cases are accompanied by subjective symptoms, such as jaundice and influenza-like symptoms [22,23]. Subjective symptoms are sometimes influenced by the patient's subjective sense. In contrast, the fluctuation of the ALT level may be a more objective index. Hofer et al. observed the natural course for at least 30 days after onset, and when serum HCV-RNA became negative during this period, the disease was resolved at a high rate, suggesting that IFN therapy should be administered to patients in whom negative conversion of HCV-RNA did not occur within 30 days [22]. Combined with our results, it might be likely that the disease resolves spontaneously in patients in whom the ALT level followed the monophasic course, as well as in those in whom the disease is symptomatic and negative conversion of HCV-RNA occurs in the early stage.

As the results of IFN therapy, the SVR rate was 80.3% (57/71) as shown in Table 4. Our present study, albeit retrospective analysis, revealed that therapy initiated within 24 weeks was the only factor related to the SVR in both univariate and multivariate analysis (Table 5). In the randomized controlled study by Hwang et al., the factor related to SVR was the HCV-RNA level before initiation of therapy [9]. However, there were only 33 patients, which may have led to a result different from our results. On the other hand, Nomura et al. recently performed a randomized controlled trial in patients with acute hepatitis C, and their results demonstrate that the SVR rate was significantly higher in the early-intervention group (IFN therapy was initiated 8 weeks

after the onset) than in the late-intervention group (IFN therapy was initiated after 1 year observation from the onset) (87% versus 40%) [24]. Otherwise, Gruner et al. prospectively investigated the T-cell dynamics in patients with acute hepatitis C, and found that activity of HCV-specific IFN- γ -producing T cells started to decrease 24 weeks after onset [25]. In addition, T cell actions have been reported to be important for elimination of HCV in the early stage of infection [26–30], and the defective functions of HCV-specific T cells might contribute to viral persistence in chronically infected patients [31]. It is interesting that our results support their reports.

Next, we evaluated the optimal timing of initiation of therapy within 24 weeks. In our previous study, we administered therapy after observation of the course for about 4 weeks when signs of the chronic hepatitis began to appear, not immediately after the onset, and obtained good results [32,33]. Licata et al. investigated the optimum timing of IFN therapy by meta-analysis [34]. Their analysis shows that delaying therapy 2 months after the onset of the disease does not affect the efficacy of treatment, therefore, they suggest that patients should be treated within 60 days from the onset to avoid the unnecessary treatment of affected patients who would spontaneously recover. In our study, the highest SVR rate was obtained in the group treated 9–12 weeks after onset of symptoms as shown in Fig. 1, which was consistent with their analytical results.

The SVR rate obtained by combination therapy with Pegylated-IFN (Peg-IFN) and ribavirin for chronic hepatitis C was 30–54% [35–37], but for acute hepatitis C, the therapeutic result was good even when IFN was administered alone. To elucidate this difference, it may be important to investigate not only the T-cell dynamics but also viral genome in various aspects [7]. In our present study, no patients were treated with Peg-IFN. Recently, the efficacy of Peg-IFN monotherapy with acute hepatitis C has been reported. Santantonio et al. evaluated the delaying Peg-IFN therapy, targeting sixteen patients who failed to spontaneously clear the virus within 12 weeks from the onset. They reported that 15/16 patients (94%) showed SVR [38]. Since the highest SVR was obtained in the group treated 9–12 weeks after onset in our study, it is important to start the IFN therapy in optimal timing regardless of the kind of IFN. The high SVR has been obtained by IFN monotherapy, so that, it is necessary to investigate whether ribavirin should be administered concurrently with IFN.

In conclusion, the major sources of infection of acute hepatitis C in Japan were the medical procedure and accidental needlestick. The disease may be likely to resolve spontaneously in patients in whom fluctuation of the ALT level follows the monophasic course. The SVR rate was significantly higher in the group treated with IFN within 24 weeks after the onset of symptoms than in the group treated after 25 weeks. In cases of acute hepatitis C, it is desirable to administer IFN at least within 24 weeks when the ALT level starts to follow a multiphasic course.

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A Decrease in AFP Level Related to Administration of Interferon in Patients with Chronic Hepatitis C and a High Level of AFP

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It is known that there is a very high incidence of hepatocellular carcinoma (HCC) among patients with type C chronic hepatitis and cirrhosis, and α -fetoprotein (AFP) has been widely used as a diagnostic marker for HCC. However, there are some patients showing continuous high AFP values but no evidence of HCC, and some studies have defined such patients as a high-risk group for HCC. In vitro study has shown that interferon (IFN) inhibits cell proliferation and enhances apoptosis as well as specific cytotoxic T lymphocytes against HCC, resulting in direct anticancer actions. In this study, we investigated the effect of IFN on AFP changes in chronic hepatitis C patients. Of 40 patients with chronic hepatitis C in whom diagnostic imaging confirmed the absence of HCC, 24 patients showed high pretreatment AFP values (high AFP group: AFP level > 10 ng/dl; mean \pm SD, 46.3 \pm 41.5 ng/dl) and 16 showed low pretreatment AFP values (low AFP group: pretreatment AFP level \leq 10 ng/dl; mean \pm SD, 5.3 \pm 2.2 ng/dl). Pretreatment clinical parameters were statistically evaluated in relation to the AFP value. In the high AFP group, the platelet count, albumin level, and prothrombin (%) were significantly lower ($P = 0.047$, $P = 0.0002$, and $P = 0.044$, respectively), suggesting that AFP value increases with advancing liver disease. Subsequently 27 patients were administered IFN (IFN group), and the remaining 13 patients were administered Stronger Neominophagen C (SNMC), a glycyrrhizin preparation (SNMC group), as a control group receiving liver-protective therapy. Alanine aminotransferase was reduced in both the IFN and the SNMC group (mean, 132.56 to 60.07 mg/ml [$P < 0001$] and 147.85 to 56.23 mg/ml [$P = 0.0240$], respectively). AFP was significantly reduced in the IFN group (mean, 30.03 to 12.65 ng/ml; $P = 0.0034$), but there was no significant change in AFP in the SNMC group (mean, 29.70 to 39.17 ng/ml). AFP is useful for diagnosing HCC; however, some patients show a persistently high AFP level in the absence of HCC, and these patients have been described as a high-risk group for HCC. In this study, we found that IFN therapy but not SNMC universally reduced the AFP baseline. Since AFP is a significant predictor for HCC, therapeutic strategies for hepatitis C, e.g., long-term low-dose IFN treatment, may reduce hepatocarcinogenesis.

KEY WORDS: hepatitis C; interferons; hepatocellular carcinoma; α -fetoprotein.

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Recently, combination therapy with pegylated interferon (IFN) and ribavirin for 48 weeks has achieved viral eradication in 54 to 56% of patients, and the occurrence of hepatocellular carcinoma (HCC) was prevented in these responders (1, 2). For nonresponders to IFN therapy, liver-protective therapy, such as oral administration of

ursodeoxycholic acid or intravenous injection of Stronger Neo-minophagen C (SNMC), is commonly performed in Japan, and it is considered that these treatments may delay the progression of liver disease (3, 4). SNMC is a glycyrrhizin preparation that exhibits potent anti-inflammatory actions and has been used to treat allergic diseases and hepatitis in Japan for centuries. However, this agent is not considered to have any antiviral or anticancer ability (5), while IFN is considered to have antiviral, anti-inflammatory, and anticancer effects, and is employed in clinical practice to treat certain types of cancer, such as germ cell tumor and RCC (6, 7).

α -Fetoprotein (AFP) has been widely used as a diagnostic marker for HCC. However, there are some patients with a high AFP baseline but no evidence of HCC, although some papers have reported that AFP is a significant predictor of HCC in such patients (8, 9). This study investigated the clinical characteristics of such patients with a high AFP baseline and assessed the effect of IFN administration in terms of AFP changes, since AFP is suggested to be an important risk factor for HCC.

METHODS

Forty patients with type C chronic hepatitis and compensatory liver cirrhosis patients who were being followed at Kurume University Medical Center were retrospectively investigated. All patients were confirmed to be positive for serum hepatitis C virus (HCV)-RNA by polymerase chain reaction (PCR). HBs-Ag-positive, autoimmune, alcoholic, and drug-induced hepatitis patients were excluded from the study. Furthermore, the absence of HCC was confirmed by abdominal ultrasonography (US) or dynamic computed tomography (CT) in all subjects.

According to the pretreatment AFP value, the 40 subjects were divided into two groups: the high AFP group (AFP > 10 ng/dl; *n* = 24) and the low AFP group (AFP ≤ 10 ng/dl; *n* = 16). Then the pretreatment clinical background parameters were statistically investigated using the Mann-Whitney *U*-test and chi-square test to compare the high and low AFP groups.

These 40 subjects were divided into two groups, the IFN group (*n* = 27) and the SNMC group (*n* = 13). Six million units of recombinant IFN α -2b was injected intramuscularly three times a week or more in the IFN group. SNMC was administered intravenously three times a week at a dose of 40 to 100 ml in the SNMC group. Both alanine aminotransferase (ALT) and AFP values after 4 weeks of treatment were compared with the pretreatment values. Paired *t*-test was used, and *P* < 0.05 was regarded as significant.

RESULTS

Clinical Characteristics in Patients with High AFP Baseline (High AFP) vs. Low AFP Group. There were no significant differences in age, gender, ALT level, HCV genotype, or HCV-RNA level between the high and the low AFP groups; however, in the high AFP group, the platelet count, albumin level, and prothrombin (PT) value were significantly lower (*P* = 0.0014, *P* = 0.0026, and *P* = 0.0041) (Table 1). These results suggest that the AFP level increases with the progression of liver disease.

Pretreatment Backgrounds in IFN and SNMC Treatment Groups. There were no significant differences in the pretreatment background parameters such as AFP value, age, gender, ALT value, platelet count, albumin level, PT (%), and HCV-RNA level between the two groups (Table 2). Fourteen of the 27 IFN-treated patients (52%) showed a high pretreatment AFP value (>10 ng/ml), and 9 of the 13 SNMC-treated patients (69%) showed a high pretreatment AFP value (>10 ng/ml).

ALT Changes in IFN and SNMC Treatment Groups. With respect to changes in the ALT level, the AFP level was significantly decreased in the IFN group (132.6 ± 72.7 to 61.1 ± 43.3 U/L; *n* = 27; *P* < 0.0001). In the SNMC group, ALT levels were also significantly decreased (149.4 ± 17.2 to 83.0 ± 57.7 U/L; *n* = 12; *P* = 0.019) (Figure 1).

AFP Changes in IFN and SNMC Treatment Groups. As for AFP changes, the AFP value was significantly

TABLE 1. PRETREATMENT CLINICAL CHARACTERISTICS ACCORDING TO AFP VALUE

	High AFP (n = 24) (AFP > 10 ng/ml)	Low AFP (n = 16) (AFP ≤ 10 ng/ml)	P value
AFP (ng/ml)	46.264 ± 41.534	5.348 ± 2.229	—
Age (yr)	55.875 ± 9.252	52.938 ± 12.179	0.3914
Gender (M/F)	14/10	12/4	0.2790
ALT (U/L)	144.333 ± 88.122	125.813 ± 83.818	0.5108
PLT (×10 ⁴ /μl)	11.421 ± 4.997	14.550 ± 4.030	0.0467*
Albumin (g/dl)	3.617 ± 0.444	4.138 ± 0.238	0.0002*
PT (%)	72.368 ± 11.923	80.237 ± 10.796	0.0439*
HCV-RNA (KIU/mL)	472.667 ± 286.404	463.067 ± 323.334	0.9257

Note. Mann-Whitney *U*-test or chi-square test was used. *P* < 0.05 was considered significant.

Values are expressed as mean ± SD.

TABLE 2. PRETREATMENT PATIENT PROFILES IN THE SNMC AND IFN GROUPS

	SNMC (n = 13)	IFN (n = 27)	P value
AFP (ng/ml)	29.970 ± 35.229	30.030 ± 39.643	0.9798
Age (yr)	54.308 ± 10.427	54.889 ± 10.685	0.8719
Gender (M/F)	9/4	17/10	0.6071
ALT (U/L)	147.846 ± 110.816	132.556 ± 272.702	0.6039
Platelets (×10 ⁴ /μl)	11.015 ± 6.244	13.441 ± 3.870	0.1387
Albumin (g/dl)	3.738 ± 0.568	3.867 ± 0.408	0.4185
PT (%)	72.615 ± 13.775	77.615 ± 10.887	0.2607
HCV-RNA (KIU/mL)	502.900 ± 299.403	455.500 ± 302.124	0.6752

Note. Mann-Whitney *U*-test or chi-square test was used. *P* < 0.05 was considered significant.

Values are expressed as mean ± SD.

decreased in the IFN group (53.0 ± 44.3 to 20.3 ± 26.7 ng/ml; $n = 14$; $P = 0.0023$). Interestingly, all 27 IFN-treated patients showed a decrease in AFP value regardless of response to treatment. However, there was no significant change in the AFP value after SNMC administration (31.1 ± 36.4 to 39.0 ± 46.5 ng/ml; $n = 9$; $P = 0.11$) (Figure 2). Mean AFP value was slightly increased in the SNMC group.

DISCUSSION

AFP is a fetal protein that is not normally present in the serum of adults and is commonly used as a tumor marker for HCC. However, serum AFP is also elevated during pregnancy and in chronic hepatitis patients (10, 11). In this study, a considerable number of type C chronic hepatitis and compensated cirrhosis patients demonstrated persistently elevated AFP levels in the absence of HCC. In addition, the AFP level decreased significantly after IFN

administration. Furthermore, the AFP decrement was universally observed regardless of treatment response to IFN therapy. Transient AFP elevation has been observed after a rise in transaminase in acute hepatitis and fulminant hepatitis (12–14). This type of AFP elevation is explained as a result of hepatocyte regeneration accompanied by necroinflammatory change. In this study, AFP was not changed in the SNMC group despite significant improvement in transaminase, suggesting that the AFP elevation was not caused by hepatocyte regeneration in chronic hepatitis patients.

AFP production is supposed to regulate the transcription level of hepatocytes (15). Among HCV-infected patients, the HCV-coding core protein is regarded to be one of the proteins responsible for hepatocarcinogenesis, up-regulating several molecules resulting in activation of the cell cycle and cell proliferation at the transcriptional level in hepatocytes (16). The HCV-coding core protein may also upregulate AFP production at the transcriptional

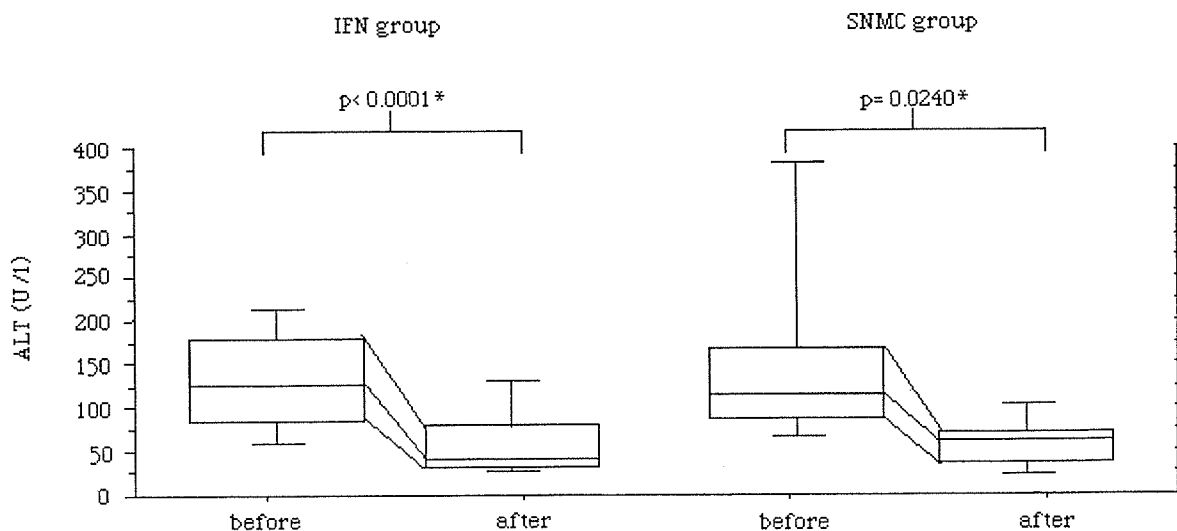


Fig 1. Changes in alanine aminotransferase (ALT) after IFN and SNMC administration. Paired *t*-test was used. **P* < 0.05 was regarded as significant.

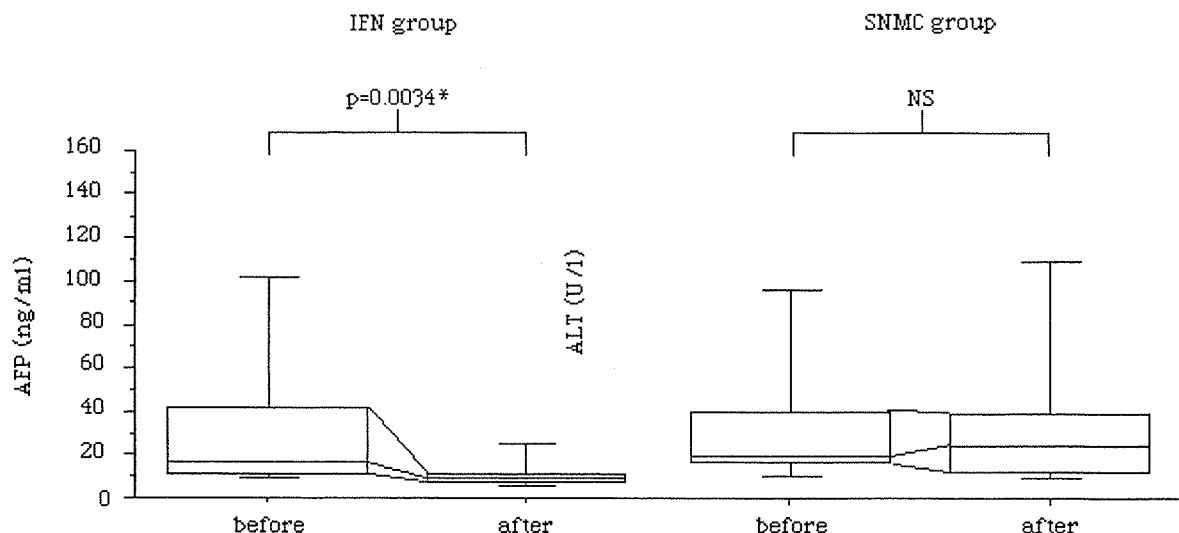


Fig 2. α -Fetoprotein (AFP) changes with IFN and SNMC administration Paired *t*-test was used. **P* < 0.05 was regarded as significant. NS, not significant.

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

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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

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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.³⁻⁶ 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%)	—	—