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Efficacy of interferon monotherapy in young adult patients with chronic hepatitis C virus infection

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Background. Suppression of the progression to cirrhosis and hepatocellular carcinoma is important, especially for young hepatitis C virus (HCV)-infected patients. The aim of this study was to analyze the response to interferon (IFN) monotherapy in young HCV patients. ***Methods.*** Between 1989 and 2002, 1021 anti-HCV-positive patients hospitalized at Toranomon Hospital received IFN monotherapy. Among these patients, 144 were ≤35 years of age, while the remaining 877 were 36–73 years old. We retrospectively identified 209 patients with known dates of blood transfusion (i.e., start of HCV infection) among the 1021 patients. IFN treatment lasted 6 months. ***Results.*** HCV RNA level ($P < 0.001$), HCV genotype ($P < 0.001$), age ($P < 0.001$), and liver histology ($P = 0.01$) were identified as determinants of the response to IFN monotherapy in 1021 patients. Moreover, in patients with high viral load and genotype 1b, the sustained virological response (SVR) rate was significantly higher in those aged ≤35 years than in older patients ($P < 0.001$). In patients with genotype 1b with known date of blood transfusion, a longer duration of infection negatively influenced the SVR rate. In the 209 patients, multivariate analysis identified HCV RNA level ($P < 0.001$), age ($P = 0.002$), and duration of infection ($P = 0.049$) as determinants of SVR. ***Conclusions.*** The response of IFN monotherapy is better in patients aged ≤35 years than in older patients, probably because of mild stage histology, the effect of host-related factors, and shorter period of infection. Long-term IFN monotherapy may be suitable for young women who desire to become pregnant or those with anemia.

Key words: HCV, interferon therapy, young patients

Introduction

Hepatitis C virus (HCV) frequently causes persistent infection and leads to chronic hepatitis, liver cirrhosis, and even hepatocellular carcinoma (HCC).[1–4] Several studies have reported the effectiveness of interferon (IFN)-based therapy in patients with HCV, particularly with regard to clearance of HCV, normalization of serum alanine aminotransferase (ALT), and reduction of the incidence of HCC.[5–7]

The number of new cases of HCV infection caused by transfusion of infected blood components is decreasing in Japan.[8] However, such cases are still being reported in the United States and other Western countries.[9,10] One of the reasons for this difference is intravenous drug use and the popularity of tattooing. The same trend, however, has been recently reported in Japan.[8] In this regard, it is important to reduce the chance of development of liver cirrhosis and HCC, especially in young infected patients. Accordingly, we believe it is important to evaluate IFN-based therapy in young patients with HCV. Currently, the combination therapy of interferon and ribavirin is widely used for patients with HCV.[11,12] However, ribavirin is reported to be harmful for pregnant and young patients, and sometimes these groups refuse to receive such combination therapy.[13,14]

The present retrospective study was designed to analyze the efficacy of IFN monotherapy in young (≤35 years of age) patients with HCV, because only a few such studies have been reported.[15,16]

Patients and methods

Patients

Between 1989 and 2002, 1021 anti-HCV-positive patients were hospitalized at Toranomon Hospital, Tokyo,

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Japan, and received first IFN monotherapy. Patients infected with both HCV and hepatitis B virus (HBV) or hepatitis A virus (HAV) or those with autoimmune diseases, previous IFN treatment for hepatitis, history of heavy alcohol abuse, drug abuse, herbal remedies, liver cirrhosis or HCC on ultrasonography, coexisting cardiac, renal, or pulmonary endocrine conditions were excluded from this study. We also retrospectively identified 209 patients among these 1021 patients for whom the day they had received the blood transfusion was known. The duration of HCV infection was calculated from the day the blood transfusion was received.

Histopathological examination of liver biopsy specimens

The baseline histopathology of chronic hepatitis was classified into four stages according to the extent of fibrosis: stage 0 (F0), no fibrosis; stage 1 (F1), periportal expansion; stage 2 (F2), portoportal septa; and stage 3 (F3), portocentral linkage or bridging fibrosis.[17] No patients with liver cirrhosis (F4) were included in this study.

Serum HCV-RNA marker

Qualitative analysis of HCV-RNA was performed using a branched DNA probe assay (bDNA probe assay, version 2.0; Chiron, Dai-ichi Kagaku, Tokyo, Japan) and a polymerase chain reaction (PCR)-based assay using the protocol provided by the manufacturer (Amplicor HCV Monitor assay version 2.0, Roche Diagnostics, Tokyo, Japan). HCV genotype was classified by PCR, using a mixture of primers for six subtypes known to exist in Japan, as reported previously.[18]

Interferon therapy

Patients received 3 to 18 Mega Units (MU) of IFN-α or -β (Sumiferon, Sumitomo Pharmaceutical, Osaka, Japan; Canferon A, Takeda Chemical Industries, Osaka, Japan; Intron A, Schering-Plough, Osaka, Japan; or Feron, Toray, Tokyo, Japan). The period of IFN treatment was 6 months. After discontinuation of therapy, all patients were followed up for at least an additional 6-month period. A sustained virological response (SVR) was defined as negative HCV-RNA by PCR at 6 months after the completion of IFN therapy.

Statistical analysis

Differences between groups were examined for statistical significance using the Mann-Whitney U test and a χ-squared test where appropriate. Independent predictive factors associated with a SVR to IFN treatment were studied using a stepwise Cox regression analysis. The following seven potential predictors were assessed in this study: HCV genotype (1b vs. other than 1b), HCV RNA level (high vs. low; a high virus quantity was defined as >100 Kiu/ml or >1 Meq/ml; other values were defined as a low virus quantity), liver histology (F1 vs. F2 or F3), duration of infection (≤5 vs. >5 years), age (≤35 vs. >35 years), total dose of IFN (≤624 vs. >624 MU), and sex (M or F). All factors found to be at least marginally associated with SVR to IFN treatment ($P < 0.15$) were entered into a multiple logistic regression. The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relative risk confidence. All analyses described above were performed using the SPSS program (version 7.5, SPSS, Chicago, IL, USA).

Results

Efficacy of interferon monotherapy

The SVR rate was 23.6% (134 of 569 patients) of patients with genotype 1b, 70.5% (206/292) of those with genotype 2a, and 48.3% (43/89) of those with genotype 2b. In the high virus load group, the SVR rates were 11.3% (49/432) for patients with genotype 1b, 52.9% (83/157) for those with genotype 2a, and 39.7% (29/73) for those with genotype 2b. In the low virus load group, the SVR rates were 62.0% (85/137) for patients with genotype 1b, 91.1% (123/135) for those with genotype 2a, and 87.5% (14/16 patients) for those with genotype 2b.

Multivariate analysis of predictive factors for response to IFN monotherapy

We explored the predictive factors for response to IFN monotherapy in 1021 patients, and the following variables were entered into the model and could not be removed: HCV RNA level ($P < 0.001$), HCV genotype ($P < 0.001$), age ($P < 0.001$), and liver histology ($P = 0.01$).

Comparison of virological response to IFN between patients ≤35 and >35 years old

Next, we examined the difference between patients aged ≤35 years ($n = 144$) and >35 years ($n = 877$) (Table 1). There was no significant difference in the sex ratio, total dose of IFN, genotype classification, or viral load between the two age groups. With regard to liver histology, more patients aged ≤35 years were classified as mild stage (F1) than those in the other group ($P < 0.001$). We also examined SVR rates according to age (≤35 and >35

Table 1. Comparison between patients ≤35 years old and >35 years old treated with interferon monotherapy

	≤35 years	>35 years	*P* value
Number	144	877	
Age (years)	31 (16–35)	52 (36–73)	
Sex (male/female)	105/39	585/292	NS
HCV-RNA level (high/low)[a]	99/45	602/275	NS
Liver histology (F1/F2/F3/N)[b]	124/18/1/1	513/313/28/23	<0.001
Total dose of interferon (MU)	624 (216–1440)	624 (216–1680)	NS
Genotype (1b/2a/2b/N)[c]	85/38/17/4	484/254/72/67	NS

Data values are expressed as medians with ranges in parentheses unless indicated otherwise
HCV, hepatitis C virus; NS, not significant
[a]High, high viral load ≥100 kiu/ml or ≥1 Meq/ml; low, low viral load <100 kiu/ml or <1 Meq/ml
[b]Liver fibrosis classified as F0, no fibrosis; F1, periportal expansion; F2, portoportal septa; F3, portocentral linkage or bridging fibrosis; N, liver biopsy was not performed
[c]N, not done

Table 2. Comparison of sustained virological response to interferon monotherapy between patients ≤35 years old and >35 years old

	≤35 years	>35 years	*P* value
Low viral load group			
1b	14/22 (63.6%)	71/115 (61.7%)	NS
2a	17/17 (100%)	106/118 (89.8%)	NS
2b	4/4 (100%)	10/12 (83.3%)	NS
High viral load group			
1b	18/63 (28.6%)	31/369 (8.4%)	<0.001
2a	16/21 (76.1%)	67/136 (49.2%)	0.019
2b	9/13 (69.2%)	20/60 (33.3%)	0.0067

years), genotype (1b, 2a, 2b), and viral load (high, low) (Table 2). In the low viral load group, there was no significant difference in SVR rate between the ≤35 and >35 age groups. On the other hand, in the high viral load group, the SVR rate was higher in the younger group than in the older group. In particular, the SVR rate was significantly higher in the ≤35 years group with a high viral load and genotype 1b compared with the respective patients aged >35 years of age ($P < 0.001$).

Relationship between duration of infection and virological response to IFN

Figures 1 and 2 show the relationship between duration of infection and SVR rate for patients with genotype 1b and genotype 2 (2a or 2b) among 209 patients whose infection duration could be assessed based on their history of blood transfusion. For the group with genotype 1b, the longer the duration of the infection, the lower the SVR rate was (Fig. 1). However, no such relationship was identified for the group with genotype 2 (Fig. 2). The duration of infection in patients aged >35 years was longer than that in patients aged ≤35 years (>35, 29 years (median); ≤35, 12 years (median), $P < 0.001$).

For the 209 patients, we explored the predictive factors for response to IFN monotherapy. Univariate analysis showed the following four factors to significantly influence the response to IFN monotherapy: genotype ($P < 0.001$), HCV RNA level ($P < 0.001$), age ($P = 0.019$), and liver histopathology ($P = 0.035$). Since the variables could be mutually correlated, a multivariate analysis was performed. In the last step, the following variables were entered into the model and could not be removed: genotype ($P < 0.001$), HCV RNA level ($P < 0.001$), and age ($P < 0.001$) (Table 3).

We also explored the determinants of the response to IFN monotherapy among the patients with genotype 1b (Table 3). Univariate analysis identified the following four factors as having significantly influenced the response to IFN monotherapy: HCV RNA level ($P < 0.001$), age ($P < 0.001$), duration of infection ($P = 0.014$), and liver histopathology ($P = 0.036$). Since the variables could be mutually correlated, a multivariate analysis was performed. The analysis identified the following three variables as significant and independent determinants of the response to IFN: HCV RNA level ($P < 0.001$), age ($P = 0.002$), and duration of infection ($P = 0.049$; Table 6). Next, we explored the predictive factors

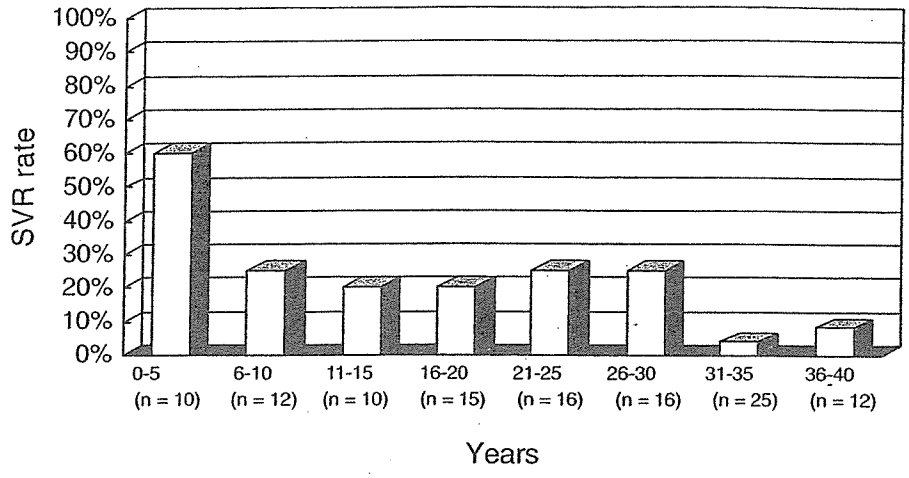

Fig. 1. Relationship between duration of infection and sustained virological response (*SVR*) rate in patients with genotype 1b. The SVR rate was inversely related to the duration of infection. The duration of infection was calculated from the day that the blood transfusion, presumed to have caused the infection, was received

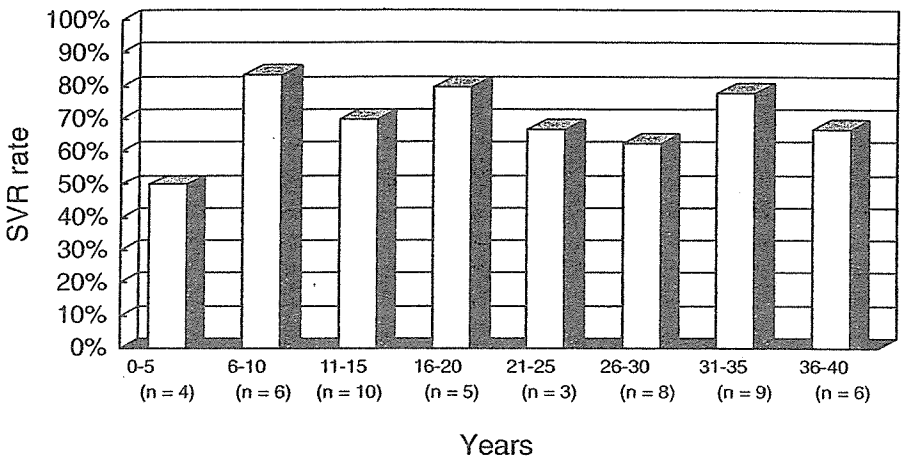

Fig. 2. Relationship between duration of infection and SVR rate in patients with genotype 2. The relationship was not significant in these patients

Table 3. Independent variables that contributed to a complete response to interferon monotherapy among 209 patients with known day of receiving blood transfusion, by multivariate analysis

Variable	Multivariate odds ratio	95% CI	*P* value
Multivariate analysis of all patients ($n = 209$)			
HCV RNA level (high vs. low)	14.618	6.293–33.952	<0.001
Genotype (1b vs. 2a, 2b)	6.573	2.938–14.705	<0.001
Age (≤35 vs. >35 years)	5.416	2.130–13.772	<0.001
Multivariate analysis of patients with genotype 1b ($n = 125$)			
HCV RNA level (high vs. low)	10.120	3.467–29.540	<0.001
Age (≤35 vs. >35 years)	4.944	1.802–13.566	0.002
Duration of infection (≤5 vs. >5 years)	4.467	1.005–19.859	0.049
Multivariate analysis of patients with genotype 2a and 2b ($n = 78$)			
HCV RNA level (high vs. low)	12.089	3.234–45.196	<0.001

95% CI, 95% confidence interval

for the response to IFN monotherapy in patients with genotypes 2a and 2b (Table 3). Among the seven factors examined in univariate analysis, only HCV RNA load was identified as a significant and independent determinant of the response to IFN ($P < 0.001$; Table 3).

Discussion

It has been reported that HCV RNA level, HCV genotype, and liver histology are important determinants of the response to IFN monotherapy in patients with

HCV.[19–21] A few reports also highlight the importance of age in the response to therapy.[22] In our study, a multivariate analysis of predictive factors for response to IFN monotherapy among 1021 patients showed the following variables to be associated with SVR: HCV RNA level, HCV genotype, age, and liver histology. Our study also confirmed the importance of age for achieving a SVR. The latter result is different from that reported by other investigators, and the difference may be explained by the relatively large number of young adult patients (144 patients) in our study compared with the other studies.[23]

In our study, comparison of the response to IFN monotherapy in patients with high viral load according to age showed that the SVR rate of patients aged ≤35 years was significantly higher than that for those >35 years old. In particular, the SVR rate of patients with high viral load and genotype 1b aged ≤35 years was higher than the respective patients aged >35 years. However, no such differences were noted in patients with a low viral load. Thus, in patients with a low viral load, the higher efficacy of IFN may reflect the effect of the drug on virus-related factors and not host-related factors such as age. Why is the SVR rate in patients ≤35 years of age higher than in those >35 years old? One reason is probably liver histology; the liver histology of many young patients was at the F1 stage. Other reasons include the effect of host-related factors and a shorter period of infection, judging from the reported high SVR rate to IFN treatment for acute hepatitis C.[24–27] Considering these reports, we investigated in the present study the efficacy of treatment in patients with known duration of HCV infection.

In the present study, we analyzed patients with a blood transfusion history on the assumption that those patients were infected with HCV by blood transfusion. Among such patients, multivariate analyses also revealed that HCV RNA level, genotype, and age were important determinants of the response to IFN monotherapy. Moreover, the duration of infection and age were independent determinants of the response to IFN monotherapy among patients with genotype 1b.

With regard to the influence of host factors, the biological activity of IFN is mediated, at least in part, by the induction of intracellular antiviral proteins, such as 2′–5′ oligoadenylate synthetase (2′–5′ OAS), dsRNA-activated protein kinase (PKR), and MxA protein.[28,29] It is possible that a larger number of such intracellular antiviral proteins are induced in young patients compared with in older patients. Further in vitro studies are warranted to compare the type and number of antiviral proteins that are induced by IFN therapy in young and old patients.

IFN and ribavirin combination therapy is widely used for the treatment of patients with chronic hepatitis C infection. Such therapy has been reported to increase significantly the SVR rate compared with IFN monotherapy.[11,12] For IFN and ribavirin combination therapy, however, little is known about the effect of age on SVR. In this regard, ribavirin therapy is reported to induce embryonic malformations.[13,14] Therefore, there is an ethical problem with regard to the use of IFN and ribavirin combination therapy, at least in pregnant patients. It is also difficult to recommend the combination therapy for patients with anemia, because such therapy is reported to induce anemia in some patients.[30,31] In these patients, IFN monotherapy is the first choice for treatment. Recent studies reported good SVR rates for IFN monotherapy over 2 years.[32–34] Thus, it is recommended that young HCV patients who must avoid IFN and ribavirin combination therapy should receive long-term IFN monotherapy.

At present, new cases of HCV infection are diagnosed particularly among those who take drugs intravenously and obtain tattoos.[8–10] The spread of intravenous drug use and tattoos among young people is worrisome in Japan and other countries. These trends are expected to be associated with an increase in the number of young HCV-infected patients in the future. To prevent the development of liver cirrhosis and HCC in such patients, early introduction of IFN-based therapy is important.

In conclusion, we have demonstrated in the present study that the response to IFN monotherapy of patients aged ≤35 years is better than that of older patients. Young women who do not intend to become pregnant should be treated with IFN and ribavirin. On the other hand, young women who plan to become pregnant and/or have anemia, should receive long-term IFN monotherapy.

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Efficacy of Interferon Therapy in Elderly Patients with Chronic Hepatitis C

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Chronic hepatitis C · Elderly patients · Interferon · Side effects

Abstract
Objective: We assessed the efficacy and safety of interferon (IFN) monotherapy in 84 elderly patients aged ≥65 years with chronic hepatitis C in a retrospective cohort study. ***Methods:*** Twenty-two of the 84 elderly patients were treated with IFN at a dose of 6 million units daily for 6–8 weeks, 18 patients were treated 2–3 times a week for 24 weeks and 44 patients were treated daily for 2–8 weeks and 2–3 times a week for 16–24 weeks. ***Results:*** A sustained virological response (SVR) occurred in 35.7% (30/84) of the patients by intention-to-treat analysis. Multivariate analysis showed that patients achieved a significant SVR when: (1) serum HCV-RNA level before IFN therapy was <100 KIU/ml ($p < 0.0001$) and (2) staging of liver fibrosis was mild ($p = 0.040$). Eleven (13.1%) patients discontinued the IFN regimen due to adverse events. Regarding factors predicting discontinuation of IFN, univariate analysis showed that patients aged >70 years were prone to drop out of therapy due to adverse events in IFN therapy ($p = 0.009$). ***Conclusion:*** Our results suggest that IFN administration is suitable for 65- to 70-year-old patients with chronic hepatitis C without genotype 1b and high virus load.

Introduction

Hepatocellular carcinoma (HCC) often occurs in patients with hepatitis C virus (HCV)-RNA-positive chronic liver disease [1]. The majority of deaths due to HCC are ascribed to hepatitis viruses, of which 70–80% (corresponding to approximately 30,000/year) are attributed to persistent HCV infection in Japan [2, 3]. The yearly incidence of HCC in patients with HCV-RNA-positive cirrhosis ranges from 5 to 7% [4–6]. In the prevention of HCC, it is important to eradicate HCV-RNA with interferon (IFN) therapy [2, 3, 7]. However, various side effects have been reported in patients treated with IFN [3]. Elderly individuals are defined by the World Health Organization as those aged >65 years, and IFN treatment is mainly given to patients with chronic hepatitis C below 65 years of age because of IFN-related side effects and safety restrictions in Japan. However, in patients with stage F3–F4 disease, progression to HCC was significantly increased compared to patients with F1–F2 liver histology [8]. Thus, to prevent HCC development, it is important to clear HCV-RNA with IFN therapy in elderly patients with stage F3–F4 disease [9]. However, only few studies have targeted IFN therapy in elderly patients with chronic hepatitis C [10–13]. We therefore assessed retrospectively the efficacy and safety of IFN monotherapy in elderly patients with chronic hepatitis C.

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Patients and Methods

Patients

The number of chronic hepatitis C patients treated with IFN monotherapy at the study hospital was 2,630 between 1989 and 2000. Of these, 84 patients met the following criteria: (1) age ≥65 years; (2) IFN administration ≤6 months; (3) alanine aminotransferase (ALT) elevation >2× the upper limits (normal range: 12–50 IU/l) within 6 months; (4) no treatment with corticosteroids, immunosuppressive agents or antiviral agents during the previous 6 months; (5) no hepatitis B surface antigens, antinuclear antibodies or antimitochondrial antibodies detectable in serum by radioimmunoassay, and (6) leukocytes >3,000/mm^3, platelet count >80,000/mm^3 and bilirubin <2.0 mg/ml. Exclusion criteria were a history of alcohol abuse or advanced liver cirrhosis (LC). Subsequently, efficacy and side effects of IFN as well as factors contributing to the eradication of HCV-RNA and the IFN-related dropout rate were assessed. Our study was approved by the institutional ethics review board of our hospital. The physician in charge explained the purpose and method of this clinical trial, as well as potential adverse reactions, to each patient, who later gave his/her informed consent for participation.

IFN Therapy

IFN treatment consisted of 3 or 6 million units of IFN-α or IFN-β given according to one of three schedules. In 22 patients, the daily dose of IFN was administered for 6–8 weeks. In another 18 patients, IFN was administered three times a week for 24–28 weeks. In the third group including 44 patients, daily IFN was administered for 2–8 weeks, followed by three times a week for 16–22 weeks.

Blood and Urine Tests

Blood samples were obtained just before and 24 weeks after IFN treatment. The samples were stored at –80°C until analyzed. Using these blood samples, HCV-RNA levels before IFN monotherapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor, version 2.0, Roche Molecular Systems) [14]. Twenty-four weeks after IFN therapy, HCV-RNA levels were analyzed by the qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/ml [15]. The HCV genotype was examined by PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [16].

Definition of Response to IFN Efficacy

The therapeutic efficacy was evaluated 24 weeks after the end of IFN therapy. A sustained virological response (SVR) to IFN therapy was defined as HCV-RNA negativity using a commercial Amplicor HCV qualitative assay (Amplicor HCV, version 2.0, Roche Diagnostic Systems, Basel, Switzerland) at two time points, 3 and 6 months after the completion of IFN therapy. Absence of SVR was defined as no response.

Liver Histology

Liver biopsy specimens were obtained percutaneously or at laparoscopy using a Tohoku-University-modified Vim Silverman needle with an internal diameter of 2 mm (Kakinuma, Tokyo, Japan). Liver histology of chronic hepatitis was classified according to the extent of fibrosis into three stages: stage 1, periportal expansion; stage 2: portoportal septa, and stage 3: portocentral linkage or bridging fibrosis. In addition to LC (stage 4), we classify four stages [17].

Table 1. Characteristics of the study patients at the commencement of IFN monotherapy

Characteristics	
Patients	84
Sex, males/females	38/46
Age[a], years	67 (65–84)
Liver fibrosis, F1/F2/F3/F4/ND	28/30/5/12/9
HCV genotype, 1b/2a/2b/others	35/33/11/5
HCV-RNA[a], MEq/ml	3.9 (<0.2–22)
AST[a], IU/l	60 (22–232)
ALT[a], IU/l	70 (21–369)
Hb[a], g/dl	13.5 (10.8–15.6)
Platelets[a] × 10^4/mm^3	14.5 (8–25.3)
WBC[a]/mm^3	4,700 (2,700–8,400)
IFN regimen, C/I/C+I	22/18/44
IFNα/IFNβ	54/30

C = 6- to 8-week continuous course; I = 24-week intermittent course; C+I = 2- to 8-week continuous course + 16- to 22-week intermittent course.

[a] Medians and ranges.

Statistical Analysis

Efficacy of IFN therapy was assessed by intention-to-treat and per-protocol analyses. Multivariate analysis (multiple logistic regression analysis) was used to establish which factors contributed to the outcome of IFN therapy. Results for each variable were transformed into categorical data consisting of two simple original numbers for multivariate analysis. $p < 0.05$ was considered statistically significant. The variables used for multivariate analysis were age, gender, liver histology, aspartate aminotransferase (AST), ALT (factors associated with patients) and HCV-RNA load and genotype (factors associated with the virus) and the methods of IFN therapy (factors associated with therapy). The SPSS software package (SPSS, Chicago, Ill., USA) was applied.

Results

Patient Characteristics

Table 1 shows the characteristics of the patients. The median age of these 84 patients was 67 years (range, 65–84 years). The IFN regimen was not randomized but decided by physician advice and patient's will.

Efficacy of the IFN Therapy

Eighty-four patients were enrolled in the present study, but 11 patients dropped out due to IFN-related side ef-

Table 2. Factors predicting SVR after IFN monotherapy by univariate analysis

Factors	Category	Odds ratio	95% CI	p value
HCV-RNA	≥1/<1 MEq/ml	1/27.60	5.83–130.78	<0.0001
HCV genotype	1b/2a, 2b	1/3.06	1.15–8.13	0.025
IFN regimen[1]	I/others	1/5.88	1.22–27.03	0.027
Liver histology	F2–F4/F1	1/3.00	1.12–8.06	0.029
Sex	female/male	1/2.12	0.86–5.23	0.104
IFN regimen	others/C+I	1/1.67	0.68–4.12	0.264
ALT	≥100/<100 IU/l	1/2.17	0.50–9.40	0.302
IFN regimen	others/C	1/1.62	0.61–4.31	0.338
AST	≥76/<76 IU/l	1/1.54	0.54–4.35	0.559
IFN	α/β	1/1.33	0.53–3.36	0.542
Age	≥68/<68 years	1/1.09	0.38–3.15	0.873

[1] C = 6- to 8-week continuous course; I = 24-week intermittent course; C+I = 2- to 8-week continuous + 16- to 22-week intermittent course; CI = confidence interval.

Table 3. Factors predicting SVR after IFN monotherapy by multivariate analysis

Factors	Category	Odds ratio	95% CI	p value
HCV-RNA	≥1/<1 MEq/ml	1/42.08	6.18–286.63	0.0001
Liver histology	F2–F4/F1	1/5.97	1.08–32.92	0.040

CI = Confidence interval.

fects. The remaining 73 patients completed the IFN therapy. SVR occurred in 35.7% (30/84) by intention-to-treat analysis.

Next we examined many factors that contributed to SVR by multivariate analysis. Univariate analysis (table 2) showed that patients achieved a significant SVR when: (1) the serum HCV-RNA level before the IFN therapy was ≤100 KIU/ml ($p < 0.0001$); (2) HCV genotype was 2a or 2b ($p = 0.025$); (3) the IFN regimen was not intermittent ($p = 0.027$), and (4) staging of liver fibrosis was mild ($p = 0.027$).

Due to the mutual correlation of these variables, multivariate logistic regression analysis was performed, using four significant variables in the model. Multivariate analysis showed that patients achieved a significant SVR when: (1) the serum HCV-RNA level before the IFN therapy was ≤100 KIU/ml ($p < 0.0001$), and (2) staging of liver fibrosis was mild ($p = 0.040$; table 3).

Table 4 shows the SVR based on virus load, HCV genotype, liver histology and the IFN regimen. In patients with a virus load ≤100 KIU/ml, genotype 2a or 2b, and liver histology of stage 1, SVR was 100% in patients with continuous IFN treatment and in those receiving the continuous + intermittent IFN regimen. On the other hand, in patients with a virus load >100 KIU/ml and genotype 1b, no patients showed SVR.

Safety of IFN Therapy

Of the 84 patients originally included in this study, 11 (13.1%) discontinued the IFN regimen due to adverse events: 5 cases due to general fatigue, 3 cases due to psychiatric disorder, and 1 patient each due to retinal bleeding, conjunctivitis, and leukopenia. These side effects occurred after 49–123 days in general fatigue, 15–141 days in psychiatric disorder, 106 days in retinal infarction, 32 days in conjunctivitis, and 30 days in leukopenia. Eight of the 11 patients stopped the IFN therapy owing to adverse events. Three patients continued IFN therapy at reduced doses. The remaining patients completed IFN therapy without severe side effects.

Cumulative Dropout Rate due to Adverse Events

Figure 1 shows the cumulative dropout rate due to adverse events of IFN. The cumulative dropout rate was

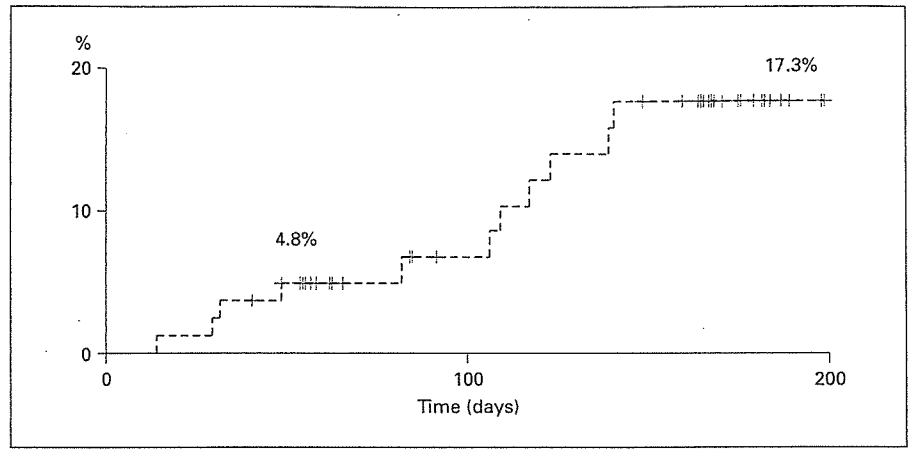

Fig. 1. Cumulative dropout rate due to adverse events during IFN therapy.

Table 4. SVR based on virus load, HCV genotype, liver histology and IFN regimen

HCV-RNA MEq/ml	HCV genotype	Liver histology	Cases	SVR based on IFN regimen[1]
<1	2a, 2b	F1	9	C; 100% (5/5), C+I; 100% (4/4)
<1	1b	F1	4	C+I; 75% (3/4)
<1	2a, 2b	F2–F4	12	I; 33.3% (1/3), C; 50% (2/4), C+I; 40% (2/5)
<1	1b	F2–F4	9	I; 0% (0/2), C; 0% (0/1), C+I; 66.7% (4/6)
≥1	2a, 2b	F1	6	C; 0% (0/3), C+I; 33.3% (1/3)
≥1	1b	F1	6	I; 0% (0/2), C; 0% (0/1), C+I; 0% (0/3)
≥1	2a, 2b	F2–F4	9	I; 0% (0/2), C; 0% (0/1), C+I; 16.7% (1/6)
≥1	1b	F2–F4	12	I; 0% (0/3), C; 0% (0/3), C+I; 0% (0/6)

[1] C = 6- to 8-week continuous course; I = 24-week intermittent course; C+I = 2- to 8-week continuous + 16- to 22-week intermittent course. Numbers of patients who showed SVR/total number of patients and percentages are shown.

4.8% 8 weeks after the initiation of IFN therapy and 17.3% at 24 weeks. We assessed factors predicting dropout based on adverse events in IFN therapy. The following factors were evaluated: sex, age, staging of liver histology, viral load, AST, ALT, Hb, platelet count, HCV-RNA level at the initiation of IFN treatment, and IFN regimen (table 5). Univariate analysis showed that patients aged >70 years were prone to dropout based on adverse events in IFN therapy ($p = 0.009$).

Discussion

Many investigators have reported IFN monotherapy and the IFN-ribavirin combination therapy to be effective for decreasing levels of ALT, reducing and eliminating HCV-RNA levels, improving liver histology and reducing the incidence of HCC in chronic hepatitis C patients [18–22]. However, clearance of serum HCV-RNA is not always attained. Factors predictive of SVR to IFN have been extensively studied, i.e. short duration of the disease, young age, absence of cirrhosis, low HCV-RNA levels and HCV genotype 2a [23–25]. Moreover, owing to IFN-related side effects or occurrence of complications, not all patients could be treated with IFN [26]. The dropout rate due to IFN-related side events might tend to increase in elderly patients. Thus, IFN therapy for chronic hepatitis C has been limited to patients aged less than 60 or 65 years. In Japanese patients >60 or >65 years, anti-inflammatory therapies, e.g. ursodeoxycholic acid or glycyrrhizin, were given. Complications related to these anti-inflammatory agents are few compared to IFN-related side effects.

Table 5. Factors predicting dropout based on IFN-related side effects

Factors	Category	Odds ratio	95% CI	p value
Age	<70/≥70 years	1/5.98	1.57–22.73	0.009
Liver histology	F2–F4/F1	1/3.76	0.46–30.56	0.216
HCV-RNA	≥1/<1 MEq/ml	1/3.01	0.797–11.39	0.104
IFN regimen	C+I/others	1/2.57	0.71–9.21	0.149
IFN	β/α	1/2.56	0.31–21.10	0.381
IFN regimen[1]	others/I	1/2.44	0.69–8.70	0.164
WBC	≥4,000/<4,000/mm^3	1/2.16	0.44–10.99	0.344
Sex	female/male	1/2.12	0.86–5.23	0.104
AST	≥76/<76 IU/l	1/1.97	0.40–3.76	0.407
Platelets	≥15/<15 ×10^4/mm^3	1/1.97	0.47–8.26	0.354
Sex	male/female	1/187	0.53–6.62	0.333
HCV genotype	2a, 2b/1b	1/1.51	0.42–5.47	0.525
Hb	≥13.5/<13.5 g/dl	1/1.51	0.36–6.33	0.574
IFN regimen	others/C	1/1.39	0.13–14.9	0.784
ALT	≥100/<100 IU/l	1/1.34	0.25–7.30	0.738

[1] C = 6- to 8-week continuous course; I = 24-week intermittent course; C+I = 2- to 8-week continuous + 16- to 22-week intermittent course; CI = confidence interval.

However, according to the statistics of the Japanese Ministry of Health, Labor and Welfare, the death rate (per 100,000 people) of HCC in Japan among people >65 years was 72.5 in 1980 and 111.1 in 2002. In the elderly, incidence rates of LC and HCC are increasing in Japan. In general, in patients aged ≥80 years with chronic liver disease, LC is the main risk factor affecting prognosis. In patients without LC, the number of liver-related deaths was lower than in patients with LC [27]. In elderly patients treated with IFN to protect the progression to LC and occurrence of HCC, life expectancy may be prolonged. Especially chronic hepatitis C patients with genotype 2a/2b or genotype 1b and lower virus load show good response to IFN therapy. Even if IFN is given at low doses, these patients could be expected to eradicate HCV-RNA and protect HCC. We, therefore, assessed the efficacy and safety of IFN therapy for chronic hepatitis C in elderly Japanese patients aged ≥65 years.

Regarding the efficacy of IFN therapy, patients who had genotype 2a or 2b, or 1b with low virus load had generally demonstrated high SVR. In the present study, elderly patients having genotype 2a/2b or genotype 1b with low virus load had high SVR. Moreover, with respect to safety of IFN therapy, the dropout rate was low in the IFN-treated elderly patients 8 weeks after initiation of IFN. We would like to recommend daily IFN therapy for 6–8 weeks in elderly patients having genotype 2a or 2b, or 1b with low virus load.

In conclusion, our results suggest that IFN administration is suitable to eradicate HCV-RNA in 65- to 70-year-old chronic hepatitis C patients without genotype 1b and high virus load.

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A Long-Term Glycyrrhizin Injection Therapy Reduces Hepatocellular Carcinogenesis Rate in Patients with Interferon-Resistant Active Chronic Hepatitis C: A Cohort Study of 1249 Patients

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To elucidate the influence of a glycyrrhizin therapy on hepatocarcinogenesis rate in interferon (IFN)-resistant hepatitis C, we retrospectively analyzed 1249 patients with chronic hepatitis with or without cirrhosis. Among 346 patients with high alanine transaminase value (twice or more of upper limit of normal), 244 patients received intravenous glycyrrhizin injection and 102 patients did not, after judgment of IFN resistance. Crude carcinogenesis rates in the treated and untreated group were 13.3%, 26.0% at the 5th year, and 21.5% and 35.5% at the 10th year, respectively ($P = .0210$). Proportional hazard analysis using time-dependent covariates disclosed that glycyrrhizin treatment significantly decreased the hepatocarcinogenesis rate (hazard ratio 0.49, 95% confidence interval 0.27–0.86, $P = .014$) after adjusting the background features with significant covariates. Glycyrrhizin injection therapy significantly decreased the incidence of hepatocellular carcinoma in patients with IFN-resistant active chronic hepatitis C, whose average aminotransferase value was twice or more of upper limit of normal after interferon.

KEY WORDS: chronic hepatitis; hepatitis C virus; glycyrrhizin; hepatocellular carcinogenesis; cancer prevention.

Until recently, hepatitis C virus (HCV) has been reported to be a causative agent of hepatocellular carcinoma (HCC) aside from hepatitis B virus (1–5). In our cohort studies of Japanese patients with HCV-related cirrhosis (5), the cumulative appearance rates of HCC at the 5, 10, and 15 years were 21.5%, 53.2%, and 75.2%, respectively. The carcinogenesis rate was higher in those patients with cirrhosis caused by HCV than in those with hepatitis B virus-related cirrhosis.

Interferon (IFN) is effective in eliminating HCV in some patients with chronic hepatitis C (6–8) and cirrhosis (9–11), and in reducing hepatocellular carcinogenesis rate through suppression of necro-inflammatory process and reduction of serum alanine transaminase (ALT). Kasahara *et al.* (6) reported that sustained normal ALT value after IFN therapy was significantly associated with a decreased hepatocellular carcinogenesis rate in patients with chronic hepatitis C. Our data (7) also demonstrated an anticarcinogenic activity of IFN in patients who attained normal ALT

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level after the therapy compared with IFN-treated patients without normalization of ALT.

Oka *et al.* (12) reported in a randomized controlled trial that a kind of medicinal herb, *Sho-saiko-to*, significantly decreased hepatic carcinogenesis rate in patients with HBsAg-negative cirrhosis. Tarao *et al.* (13) showed that HCC appearance rate was significantly higher in HCV-related cirrhotic patients with a high ALT value of 80 IU/mL or more than that of those with lower ALT value (<80 IU/mL), and also suggested that treatment of cirrhosis and prevention of HCC should be directed to suppress the necro-inflammation of HCV-related hepatitis. A glycyrrhizin-containing product, Stronger Neo-Minophagen C (SNMC; Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan), is widely used in Japan for suppression of hepatitis activity and for prevention of disease progression in patients with hepatitis B virus and HCV-induced chronic hepatitis. Glycyrrhizin has been reported to suppress hepatic inflammation with an effect to improve the elevated ALT levels and histologic findings of the liver (14–17). We reported its favorable effect on hepatocellular carcinogenesis in those patients with chronic hepatitis C who received glycyrrhizin for more than 10 years (18).

To elucidate whether glycyrrhizin suppress the carcinogenesis rate in patients with IFN-resistant chronic hepatitis C, we retrospectively assessed a cohort of 1249 patients without sustained virologic response (SVR) after IFN therapy.

PATIENTS AND METHODS

Study Population. A total of 1249 consecutive Japanese patients with chronic hepatitis C with or without cirrhosis were examined, who did not show an SVR of HCV-RNA under IFN therapy. Sera of the patients showed positive anti-HCV (second-generation anti-HCV kit, enzyme-linked immunosorbent assay, Dainabot, Tokyo, Japan), positive HCV-RNA (nested PCR), and negative hepatitis B surface antigen (HBsAg; radioimmunoassay, Dainabot). Anti-HCV and HCV-RNA were assayed using stored frozen sera at –80°C. There were 778 men and 471 women aged 18–81 years, with a median age of 53 years in the study. They were diagnosed as having liver cirrhosis by peritoneoscopy, liver biopsy, or both between 1987–2002.

All the patients had a history of receiving once or more times of IFN therapy: 1179 patients underwent IFN monotherapy only and the other 70 patients had received an IFN plus ribavirin combination therapy before the entry of this study. A total of 347 patients showed a normal ALT for at least 6 months after cessation of IFN (biochemical responders), and the other 902 patients abnormal ALT at 6 months after the end of IFN therapy. A retrospective cohort study was performed using these 1249 consecutive patients with chronic hepatitis or cirrhosis who failed to show SVR.

Glycyrrhizin Treatment. Glycyrrhizin therapy was performed using intravenous injection of SNMC. The preparation contains 0.2% (4 mg) glycyrrhizinic acid as the main active constituent, 2% (40 mg) glycine, and 0.1% (2 mg) L-cysteine in 20-mL ampoules.

Of 1249 patients with IFN-resistant chronic liver disease, 453 patients underwent glycyrrhizin injection therapy and the remaining 796 patients did not receive the therapy until the end of observation. The purpose of the introduction of the glycyrrhizin injection therapy was to suppress elevated ALT and to prevent disease progression in all the patients. Of the 453 patients, 129 (28.5%) received a daily dose of 40–60 mL of SNMC (80–120 mg as glycyrrhizin) and 324 (71.5%) received 80–100 mL (160–200 mg as glycyrrhizin). A total of 110 patients received the treatment for less than 2 years and 107 patients continued the therapy for 2–4 years, 132 patients for 4–6 years, and the remaining 104 patients for 6 years or longer. When the treatment was regarded as effective from the viewpoint of ALT levels, treatment was usually continued for a period as long as possible. As a result, a median daily dose of 100 mL of SNMC was administered 3 times a week during a median period of 4.3 years (range, 0.1–14.5 years) in the treated group.

Two (0.44%) of 453 treated patients were withdrawn from the glycyrrhizin injection therapy because of side effects: 1 because of hypertension and 1 from skin rash.

Background and Laboratory Data of Patients With and Without Glycyrrhizin Therapy. Table 1 summarizes the profiles and data of the patients at the time of diagnosis of chronic hepatitis with or without cirrhosis. The male/female ratio was not different between the 2 groups. Median age was older by 2 years in the treated group than in the untreated group ($P < .001$). Results of histologic staging of liver disease were classified according to Desmet *et al.* (19). F1stage hepatitis was found significantly more often in the untreated group than in the glycyrrhizin group ($P < .001$, χ^2 test). Both AST and ALT median levels were significantly higher in the treated group than in the untreated group ($P < .001$). HCV subtype was analyzed by the immunoserologic typing method with a commercial kit (Kokusai Diagnostic Corporation, Kobe, Japan): serologic group 1 indicated genotypes 1a and 1b, and group 2 included 2a and 2b subtypes. The rate of HCV serologic group 1 was significantly higher in the glycyrrhizin group than in the untreated group ($P = .032$).

Follow Up. Follow-up of the patients was made monthly after the judgment of IFN-resistance by monitoring hematologic, biochemical, and virologic data. Imaging diagnosis with ultrasonography (US) and/or computed tomography (CT) was made 3 or more times per year in a majority of patients with cirrhosis and once a year in patients without cirrhosis. Angiographic study was performed only when HCC was strongly suspected on US or CT.

When angiography revealed a characteristic hypervascular nodule suggesting a specific finding for HCC, no histologic examination was made in a majority of these patients. An increasing trend of tumor markers was also taken into account in establishment of the diagnosis of HCC. Microscopic examination through a fine needle biopsy was also performed in patients whose angiogram did not show a typical image of HCC.

The number of cases lost to follow-up was 121 (9.7%): 28 patients (6.2%) in the glycyrrhizin group and 93 (11.7%) in the untreated group. Because the outcomes regarding appearance of HCC were not identified in these patients, they were dealt as censored data in the following statistics (20). Death unrelated to HCC was also classified as withdrawal and regarded as a censored case. The median observation period of the total number of patients was 5.7 years with a range of 0.1–16.1 years. Because

TABLE 1. PATIENT PROFILES AND LABORATORY DATA AT TIME OF JUDGMENT OF IFN RESISTANCE

	Glycyrrhizin Group (n = *453*)	*Untreated Group* (n = *796*)	P
Demographics			
Gender (M/F)	283/170	495/301	.92
Age (y)*	54 (25–81)	52 (18–77)	<.001
Observation period (y)*	8.3 (0.1–16.1)	5.1 (0.1–13.1)	<.001
Liver histology			
F1	146 (32.7%)	502 (64.0%)	<.001
F2	193 (43.3%)	192 (24.5%)	
F3	38 (8.5%)	52 (6.6%)	
F4	69 (15.5%)	38 (4.8%)	
Laboratory data*			
Aspartic transaminase (IU/L)*	81 (19–446)	54 (11–355)	<.001
ALT (IU/L)*	122 (12–630)	83 (10–822)	<.001
HCV serologic group 1 (1a or 1b)	360 (80.2%)	582 (73.7%)	.032
Group 2 (2a or 2b)	73 (16.3%)	165 (20.9%)	
Others	16 (3.6 %)	43 (5.4%)	

*Expressed as median (range).

many patients receiving glycyrrhizin therapy migrated from the untreated group to the treated group, observation period of the untreated group was significantly shorter than that of the treated group (see Table 1). The date of the last follow-up for this study was September 1, 2003.

Statistical Analysis. Nonparametric procedures were employed for the analysis of background characteristics of the patients, including Mann-Whitney U-test and χ^2 method. HCC appearance rates were calculated from the time period between the judgment of IFN ineffectiveness and appearance of HCC in each group, using Kaplan-Meier technique (20). The differences in carcinogenesis curves were tested using the log-rank test. Independent factors associated with the appearance rate of HCC were studied using time-dependent Cox regression analysis (21). An interaction term of IFN treatment and "waiting time" to the therapy was introduced in the analysis as a time-dependent covariate. The independence of treatment factor from "waiting time" was also confirmed by log-minus-log plot of proportional hazard model. Several variables were transformed into categorical data consisting of 2–3 simple ordinal numbers to estimate each hazard ratio. All factors found to be at least marginally associated with liver carcinogenesis ($P < .15$) were tested by the multivariate Cox proportional hazard model. A P-value of less than .05 was considered to be significant. All data analysis was performed using the computer program SPSS version 11 (22).

TABLE 2. INDEPENDENT RISK FACTORS AFFECTING HEPATOCELLULAR CARCINOGENESIS

Factors	*Category*	*Risk Ratio (95 % CI)*	P
Fibrotic stage			
	F1	1	
	F2–3	2.94 (1.20–7.21)	.018
	F4 (cirrhosis)	9.21 (3.73–22.8)	<.001
Gender			
	1: Female	1	
	2: Male	2.80 (1.35–5.81)	.006
Glycyrrhizin injection			
	1: No	1	
(SNMC)*	2: Yes	0.49 (0.27–0.86)	.014

Time-dependent Cox proportional hazard analysis. *SNMC, Stronger Neo-Minophagen C (herbal medicine containing glycyrrhizin).

RESULTS

Initial Aminotransferase and Carcinogenesis Rates

Patients with and without glycyrrhizin therapy were classified into 6 categories according to average ALT value during the first year after cessation of IFN therapy: group 1, normal ALT; group 2, <1.5 times of upper limit of normal (ULN); group 3, 1.5–2 times ULN; group 4, 2–3 times ULN; group 5, 3–4 times of ULN; and group 6, >4 times ULN. Hepatocellular carcinogenesis rates were 2.5%, 5.0%, 8.1%, 11.8%, 12.0%, and 12.7% at the end of 5 years and 6.6%, 7.2%, 19.6%, 15.1%, 21.0%, and 39.3% at 10 years, respectively. (Figure 1). There was a significant statistical difference among the 6 subgroups (log-rank test, $P < .0001$). The higher the average ALT, the higher the carcinogenesis rate was.

Influence of Glycyrrhizin on Carcinogenesis in Patients With High Aminotransferase

Glycyrrhizin therapy was usually performed in patients with a high ALT value and high hepatitis activity. In this retrospective study, average ALT values were significantly different between the treated and untreated groups: group 1, normal average ALT was found in 38 among patients with glycyrrhizin therapy and in 188 among patients without therapy; in group 2, ALT <1.5 times of ULN was found in 42 and 331; in group 3, 1.5–2 times ULN in 84 and 138; in group 4, 2–3 times ULN in 143 and 92; in group 5, 3–4 times in 53 and 29; and in group 6, ALT

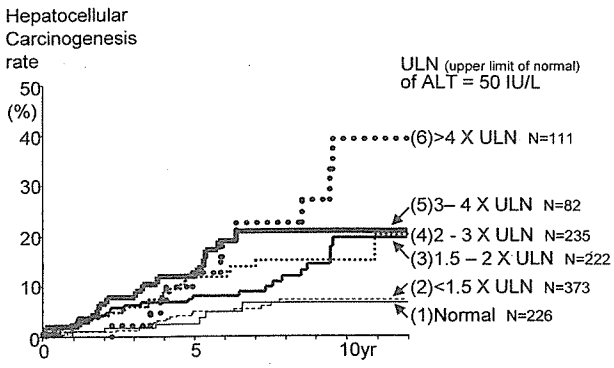

Fig 1. Carcinogenesis rates according to initial ALT values classified into six groups: (1) normal ALT, (2) <1.5 times ULN, (3) 1.5–2 times ULN, (4) 2–3 times ULN, (5) 3–4 times ULN, and (6) >4 times of ULN. The higher the average ALT, the higher the carcinogenesis rate was.

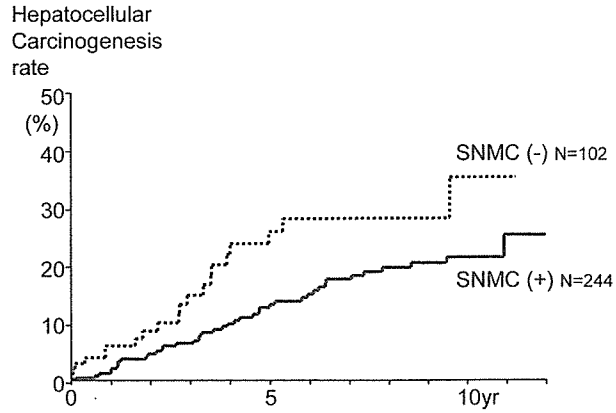

Fig 2. Carcinogenesis rates in patients with high average ALT values of twice or more of ULN, excluding those patients with biochemical responders who continued a normal ALT value at least 6 months just after IFN therapy. The carcinogenesis rate of glycyrrhizin-treated group was significantly lower than that of the untreated group (log-rank test, $P = .0210$).

>4 times ULN in 93 of the glycyrrhizin group and 18 of the untreated group. The rate of a high ALT value of twice or more of ULN in the glycyrrhizin treated group (64.2%, 289/453) was significantly higher than that of the untreated group (16.2%, 129/796).

Of the 418 patients with a high average ALT in both groups, 68 patients showed a normal ALT value for at least 6 months just after IFN therapy (biochemical response). Because biochemical response with normal ALT for a certain period after IFN was likely to affect carcinogenesis rates in those patients, biochemical responders were excluded in the following analyses about the influence of glycyrrhizin on carcinogenesis: after all, 244 patients with glycyrrhizin therapy and the 102 patients without therapy were assessed.

Cumulative hepatocellular carcinogenesis rates were calculated in these 346 patients with a high average ALT values, excluding biochemical responders from both groups. Carcinogenesis rates in the glycyrrhizin group and the untreated group were 6.5% and 13.3% at the end of year 3, 13.3% and 26.0% at the end of year 5, 17.7% and 28.3% at the end of year 7, and 21.5% and 35.5% at year 10, respectively (Figure 2). In the stratified and selected patient group, the carcinogenesis rate of glycyrrhizin-treated group was significantly lower than that of the untreated group (log-rank test, $P = .0210$).

Carcinogenesis Rates According to Hepatitis Staging

Crude carcinogenesis rates were compared between the groups, according to each hepatitis stage. In F1 stage chronic hepatitis, hepatocellular carcinogenesis rates in the glycyrrhizin group ($n = 82$) and the untreated group ($n = 32$) were 1.4% and 4.2% at year 5 and 7.0% and 12.1% at 10 years, respectively (Figure 3A). In F2–3 stage chronic hepatitis, hepatocellular carcinogenesis rates in the glycyrrhizin group ($n = 121$) and the untreated group ($n = 53$) were 14.8% and 28.4% at the end of year 5, and 21.5% and 38.6% at year 10, respectively (Figure 3B). In patients with F4 stage chronic hepatitis (cirrhosis), hepatocellular carcinogenesis rates in the glycyrrhizin group ($n = 38$) and the untreated group ($n = 15$) were 35.2% and 58.0% at the end of year 5, and 57.2% and 58.0% at year 10, respectively (Figure 3C).

In each fibrotic stage of hepatitis, carcinogenesis rates were lower in the glycyrrhizin group than in the untreated group, but statistical significance was not obtained owing to shortage of patient number in these stratified groups.

Aminotransferase Activity Before and After Glycyrrhizin Therapy

ALT values in the patients with glycyrrhizin treatment were serially assessed in those patients who began the therapy after they had shown a high average ALT value (Figure 4). Median value of ALT at the beginning of the glycyrrhizin therapy was 150 IU/L (25th percentile 120, 75th percentile 221), 72 IU/L at month 3, 70 IU/L at month 6, and 64 IU/L (25th percentile 48, 75th percentile 93) at month 12, respectively. ALT value significantly decreased after the initiation of glycyrrhizin injection therapy.

Factors Affecting Carcinogenesis Rates in Active Hepatitis and Cirrhosis

In the selected patients with active hepatitis with an average ALT value of twice ULN or higher, multivariate analysis was performed to explore associating factors with carcinogenesis, using time-dependent Cox proportional hazard model. Time between the judgment of IFN

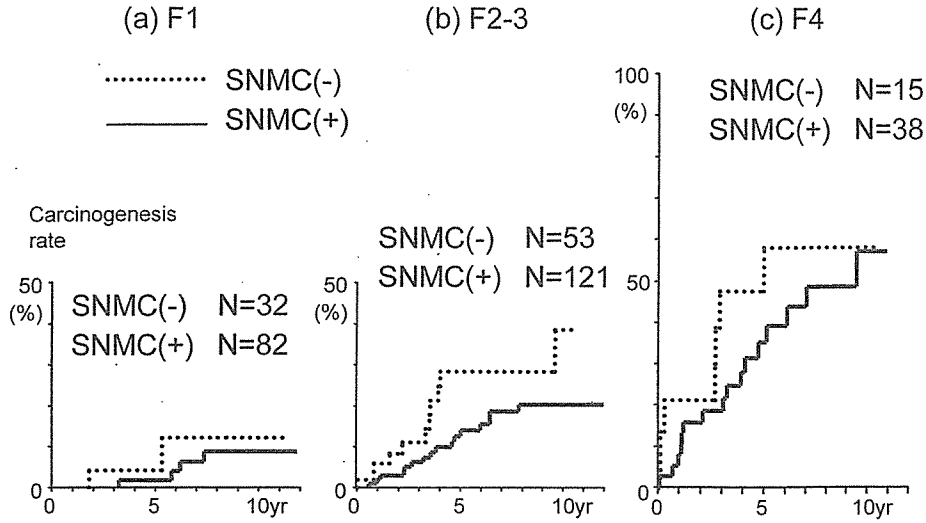

Fig 3. Carcinogenesis rates according to hepatitis staging: (a) F1 stage hepatitis, (b) F2–F3 stage hepatitis, and (c) F4 or cirrhotic stage. In each fibrotic stage of hepatitis, carcinogenesis rates were lower in the glycyrrhizin group than in the untreated group.

ineffectiveness and initiation of glycyrrhizin therapy was set as a time-dependent variable to clarify the significance of glycyrrhizin therapy in the clinical course of HCV-related chronic liver diseases. Patients with biochemical response with a normal ALT value sustained for at least 6 months after IFN therapy were also excluded from the analysis.

In multivariate analysis, following 3 factors influenced the carcinogenesis: fibrotic staging, gender ($P = .006$), and glycyrrhizin therapy ($P = .014$). When a hazard of F1 stage fibrosis for carcinogenesis was set as 1 in the model, hazard ratio of F2–F3 stage fibrosis was calculated as 2.94 ($P = .018$), and that of F4 (cirrhosis) was estimated as 9.21 ($P < .001$). Similarly, the hazard ratio for carcinogenesis of male gender was 2.80, and use of glycyrrhizin independently decreased the carcinogenesis rate in patients with active chronic hepatitis after IFN therapy. Following factors did not affect the HCC appearance rate significantly: age, association of diabetes mellitus, serologic grouping of HCV, HCV-RNA concentration, AST, ALT at the time before IFN therapy, and bilirubin.

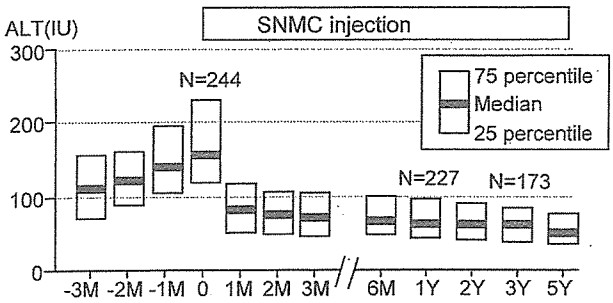

Fig 4. Aminotransferase activity before and after glycyrrhizin therapy. ALT value significantly decreased after the initiation of glycyrrhizin injection therapy.

DISCUSSION

IFN is effective in patients with chronic liver disease caused by HCV, from the viewpoints of anti-inflammatory effect and cancer prevention (6–11). Although the carcinogenesis rate is noticeably reduced when aminotransferase becomes normal with or without HCV-RNA eradication (6–8) after the therapy, the rate of normalization of ALT after IFN therapy is approximately half of patients with high viral load and group 1 HCV-subtype.

This retrospective study was undertaken to evaluate whether long-term glycyrrhizin injection therapy could decrease hepatocellular carcinogenesis rate in patients with IFN-resistant HCV-related chronic hepatitis and cirrhosis. Because it requires at least 5 years to show a statistical difference in carcinogenesis rate from hepatitis or cirrhosis between glycyrrhizin-treated and "untreated" groups, a prospective randomized trial using untreated control patients is difficult from both ethical and medical viewpoints in Japan, where glycyrrhizin injection therapy is covered by standard medical insurance and is already regarded as a usual choice of therapy as a salvaging procedure for IFN-ineffective patients. We, therefore, attempted to carry out this retrospective cohort study to prove an anticarcinogenic activity of glycyrrhizin, with a statistical adjustment using possible covariates explored in multivariate analysis.

Because glycyrrhizin injection therapy was chiefly performed for patients with a high ALT value and because cancer prevention was meaningful in just those patients with a high carcinogenesis risk with high hepatitis activity, we analyzed the role of a long-term glycyrrhizin injection therapy in the patients with a high ALT value. The treated group consisted of significantly more numbers of patients with a high ALT value of twice or more of ULN. When carcinogenesis rates were assessed only in those patients with a high ALT value of twice or more ULN excluding biochemical responders, the rate of the treated group became significantly higher than that of the untreated group ($P = .021$). The cancer preventive effect of glycyrrhizin in IFN-resistant patients was also confirmed by time-dependent Cox proportional analysis that adjusted the background features of the retrospective cohort (hazard ratio = 0.49, $P = .014$). We previously reported a study focused on the anticarcinogenic action of glycyrrhizin for patients with chronic hepatitis C, but the pilot study only demonstrated that 10 years or longer treatment with glycyrrhizin ($n = 84$) could suppress the carcinogenesis rate (18). Current study dealing with a large cohort ($n = 1249$) showed that glycyrrhizin injection therapy significantly decreased carcinogenesis rate irrespective of the length of treatment when comparison was made in a selected patient cohort with high hepatitis activity.

Although a statistically significant difference was not shown for a lack of sufficient patient number in subgroups of chronic hepatitis and cirrhosis, this study also demonstrated that glycyrrhizin was effective not only in chronic hepatitis but also in cirrhosis. Considering that liver cirrhosis generally shows a resistance to IFN treatment, our current study demonstrated encouraging results from the viewpoint of HCC prevention. When IFN therapy was attempted in 7 patients with decompensated cirrhosis by Nevens *et al.* (23), complications sometimes occurred in these patients, including variceal bleeding, aggravation of ascites or encephalopathy, development of pneumonia, and recurrence of spontaneous bacterial peritonitis or gastric ulcer bleeding. Because patients with cirrhosis usually showed lower platelet and leukocyte counts than those with chronic hepatitis and because cirrhotic patients tended to show deterioration with a large dose of IFN, glycyrrhizin therapy proved to be a useful alternative of therapy. Intermittent long-term glycyrrhizin therapy was well tolerated with withdrawal of only 2 patients (0.44%).

Because carcinogenesis is not a single-step event but a complex, multistep process, the exact mechanism of the glycyrrhizin activity in suppression of liver carcinogenesis remains unknown. One of the principal roles of long-term administration of glycyrrhizin in decreasing the carcinogenesis rate is considered to be anti-inflammatory, which blocks the active carcinogenic process of continuous hepatic necro-inflammation and cell damage. In the treated group, median ALT values markedly decreased after initiation of the glycyrrhizin injection, suggesting that pathologic process of hepatocyte necrosis or apoptosis was significantly suppressed by glycyrrhizinic acid. The importance of the action of amino acids, glycine and cysteine contained in SNMC has not been completely explained, but they have been demonstrated to suppress increased aldosterone levels that are induced by glycyrrhizinic acid. Tarao *et al.* (24) reported that high aminotransferase level resulted in an increase of an HCC recurrence rate in patients with HCC. From the viewpoint of these anti-inflammatory activities, SNMC may be considered to only postpone the time of HCC appearance in the clinical course of cirrhosis. Because the entire process of hepatocellular carcinogenesis from the initial transformation of a hepatocyte to a detectable growth of cancer is considered to take at least several years, the influence of glycyrrhizin on the carcinogenesis rate will not be evaluated in a short period. Although several reports suggested a relationship of anti-hepatitis B core antibody or hepatitis B surface antibody with carcinogenesis (25–27), we could not show the association because of insufficient available data.

Because current data were obtained from a retrospective cohort analysis, dose of glycyrrhizin per time, times of injection per week, and duration of therapy varied in each patient in the treated group. To elucidate the cancer preventive effect of glycyrrhizin therapy in active HCV-related liver disease, we should further stratify the treated patients or perform much more detailed statistical procedures. Future studies should, therefore, aim at defining the basic oncogenic mechanisms and roles of long-term administration of glycyrrhizin in carcinogenesis in patients with cirrhosis caused by HCV.

In conclusion, a long-term intermittent glycyrrhizin therapy for a few years or more successfully reduced hepatocellular carcinogenesis in patients with HCV-related chronic liver disease. A randomized control study with a larger number of cases, with or without glycyrrhizin therapy, is expected to confirm the effectiveness of this therapy.

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