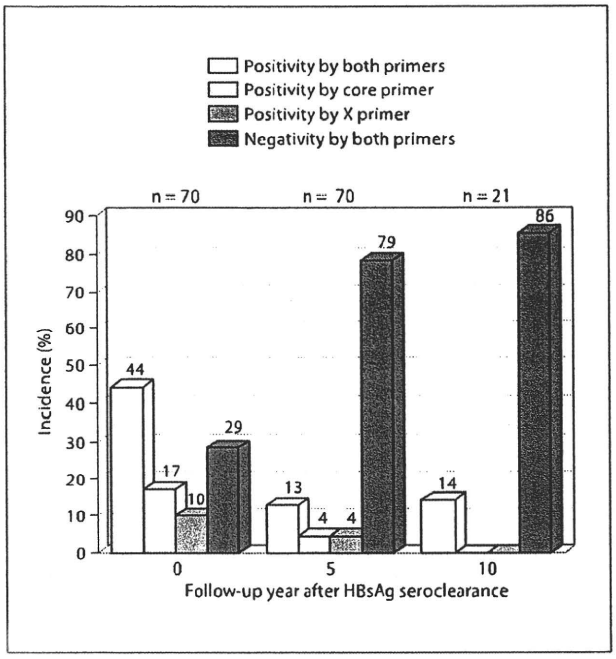

Fig. 1. Changes of detection pattern of serum HBV DNA after seroclearance of HBsAg. Negative controls: 10 healthy volunteers; positive controls: 10 patients with chronic hepatitis B; core (X): positivity indicates positive HBV DNA by nested PCR using core (X) gene primers, negativity indicates negative HBV DNA by nested PCR using core (X) gene primers.

Table 1. Characteristics of subjects at the time of seroclearance of HBsAg

Number	70
Sex (male/female)	55/15
Age, years	53 (30–82)
HBV genotype (A/B/C/D/F)	3/7/45/2/6
US (non-LC/LC)	40/30
Total protein, g/dl	7.4 (6.6–8.8)
Albumin, g/dl	4.2 (3.4–5.1)
Total bilirubin, g/dl	0.7 (0.1–1.7)
AST, IU/l	21 (11–71)
ALT, IU/l	16 (6–101)
Hb, g/dl	15.2 (12.9–17.1)
Platelets, $\times 10^4/mm^3$	17.3 (8.4–32.5)
Follow-up period after disappearance of HBs antigen, years	8.3 (5.3–23.6)

Data are numbers of patients or medians, with ranges in parentheses. ALT = Alanine aminotransferase; AST = aspartate aminotransferase; Hb = hemoglobin; US = ultrasonographic findings; LC = liver cirrhosis.

Table 2. Change of anti-HBc antibody after HBsAg seroclearance

	Follow-up year of HBsAg seroclearance		
	0	5	10
Anti-HBc antibody	14.2 ± 2.7	13.9 ± 2.2	13.3 ± 3.6
Anti-HBc antibody (200-fold dilution)	6.5 ± 4.0	1.8 ± 1.4	0.9 ± 0.7

The serum was diluted 1:200 with saline. The titer of anti-HBc antibody was determined by the chemiluminescent immunoassay method.

($p < 0.05$) were subjected to multiple logistic regressions to identify significant independent predictors. The SPSS software package (SPSS 10.0 for Windows; SPSS Inc., Chicago, Ill., USA) was used for analyses.

Results

Clinical Profiles

Table 1 shows the characteristics of the 70 patients who had seroclearance of HBsAg. The median age of the 70 patients (male 55, female 15) was 53 years. Thirty-seven patients had spontaneously cleared HBsAg. At the time of HBsAg seroclearance, 30 patients showed liver cirrhosis.

Sixty-three of 70 (87.9%) patients had normal alanine aminotransferase levels after HBsAg seroclearance. Seven patients with elevated alanine aminotransferase had 4 fatty infiltrations of the liver and 3 cases of alcohol abuse.

Changes of Anti-HBs, Anti-HBe and Anti-HBc

Table 2 shows the titers of serum anti-HBc. As regards the titer of nondiluted anti-HBc, there was no difference between the time of HBsAg seroclearance, 5 years and 10 years after HBsAg seroclearance. The titers of 200-fold diluted serum anti-HBc decreased 5 and 10 years after HBsAg seroclearance with statistical significance.

Serum HBV DNA after HBsAg Seroclearance

The detection pattern of serum HBV DNA based on the difference of HBV primers by the nested PCR is shown in figure 1. The negative controls of healthy volunteers showed negative HBV DNA with both primers. On the

Table 3. Predictive factors for the positivity of HBV DNA 5 years after HBsAg seroclearance

Factor	Category	Odds ratio	95% CI	p value
IFN therapy	−/+	1/0.58	0.14–2.32	0.438
Age, years	<60/≥60	1/1.99	0.56–7.07	0.287
Total protein, g/dl	<8/≥8	1/1.84	0.90–3.76	0.096
Liver histology	non-LC/LC	1/2.00	0.28–5.48	0.781
HBV genotype	B/C	1/0.254	0.05–1.36	0.109
Sex	male/female	1/0.23	0.03–1.88	0.169
AST, IU/l	≥38/<38	1/1.59	0.57–4.43	0.375
Platelets, $\times 10^4/mm^3$	≤20/>20	1/1.20	0.700–2.06	0.504
ALT, IU/l	≥50/<50	1/1.28	0.46–3.55	0.634

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; LC = liver cirrhosis.

other hand, the positive controls with chronic hepatitis B showed positive HBV DNA with both primers. The consistent rate of PCR detection of HBV DNA using both the X gene and core primers was 84.5% (136/161) in all. The positive rate of HBV DNA was 71.4% (50/70) at year 0, 21.4% (15/70) at 5 years and 14.3% (3/21) at 10 years by using both the X gene and core primers.

A multivariate regression analysis was used to assess the factors contributing to the positivity of serum HBV DNA: the factors examined included age, gender, histological findings, HBV genotype and IFN administration. However, there were no significant factors contributing to the positivity of serum HBV DNA (table 3).

In total 68 serum samples which showed positive HBV DNA by the nested PCR were examined by the transcription-mediated amplification and hybridization-protection assay. All the samples showed serum HBV DNA of less than 3.7 LGE/ml.

Discussion

In the present study, the detection rate of serum HBV by the nested PCR after HBsAg seroclearance was about 70% at the time of HBsAg seroclearance and about 10–20% at 5 and 10 years after seroclearance of HBsAg. The positive rate of HBV DNA decreased 5 and 10 years after HBsAg seroclearance compared to the time of HBsAg seroclearance. Moreover, the titer of anti-HBc antibody by the 200-fold dilution gradually decreased after HBsAg seroclearance. This suggests that HBV may ultimately be cleared from the serum after a long time of more than 10 years. However, this present study showed that about 10–20% of patients had serum HBV DNA levels of 50–100 copies/ml at 5 and 10 years after seroclearance of HBsAg. The remaining patients might have low levels of HBV DNA of <50–100 copies/ml. Yuen et al. [14] have reported that HBV remains in the liver even if serum HBV is shown to be negative in some patients. These findings mean that a trace of HBV remains for a prolonged period after HBsAg seroclearance.

HBV DNA replications sometimes occur after administration of steroids and/or immunosuppressive agents in patients with a small amount of residual HBV [19–21]. Our previous study suggests that steroid withdrawal therapy for HBeAg-positive patients with chronic hepatitis induces an elevation of serum HBV DNA and acute exacerbation of liver function [10]. Five of 230 HBeAg-positive patients treated with steroid withdrawal therapy showed acute exacerbation of liver function and icterus. Therefore, when the patients with serum HBsAg are given steroids and/or immunosuppressive agents, they should be carefully followed up by monitoring serum levels of HBV DNA and liver function. The present study shows that a trace of HBV remains during a prolonged period after HBsAg seroclearance. This suggests the following point: when patients with HBsAg seroclearance are treated with steroids and/or immunosuppressive agents, they should be carefully followed up by monitoring the serum level of HBV DNA and/or liver function to prevent acute exacerbation of liver impairment.

Seventy patients enrolled in the present study were not treated with steroids and/or immunosuppressive agents, so they did not show acute exacerbation during the follow-up. Moreover, these 70 patients did not show progression to decompensated liver cirrhosis and/or death due to hepatocellular carcinoma. Thus, our results suggest that even if patients with HBsAg seroclearance have a trace of

HBV DNA, they have generally a good prognosis concerning liver function.

In conclusion, as some patients also had a trace of serum HBV DNA 5 and/or 10 years after seroclearance of HBsAg, they should be carefully followed concerning administration of steroids and/or immunosuppressive agents.

Acknowledgements

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Glycyrrhizin injection therapy prevents hepatocellular carcinogenesis in patients with interferon-resistant active chronic hepatitis C

Kenji Ikeda

Department of Hepatology, Toranomon Hospital, Tokyo, Japan

Aim: There is no useful and effective treatment for patients with non-sustained response to interferon, from the viewpoint of cancer prevention. Our aim was to elucidate the influence of a glycyrrhizin therapy on hepatocarcinogenesis rate in interferon-resistant hepatitis C

Methods: We retrospectively analyzed 1249 patients with chronic hepatitis with or without cirrhosis. Among 346 patients with high alanine transaminase values of twice or more of the upper limit of normal, 244 patients received i.v. glycyrrhizin injection and 102 patients did not, after judgment of interferon resistance.

Results: Crude carcinogenesis rates in the treated and untreated group were 13.3%, 26.0% at the fifth year, and 21.5% and 35.5% at the 10th year, respectively ($P = 0.021$). Proportional hazard analysis using time-dependent covariates disclosed that fibrotic stage, gender and glycyrrhizin treatment were significantly associated with future carcinogenesis. A long-term glycyrrhizin injection therapy decreased the hepatocarcinogenesis rate (hazard ratio, 0.49; 95% confidence interval, 0.27–0.86, $P = 0.014$) after adjusting the background features with significant covariates. Cancer preventive activity was also found in a subgroup of older patients of 60 years or more.

Conclusions: Glycyrrhizin injection therapy significantly decreased the incidence of hepatocellular carcinoma in patients with interferon-resistant active chronic hepatitis C, whose average aminotransferase value was twice or more of the upper limit of normal after interferon.

Key words: cancer prevention, chronic hepatitis, glycyrrhizin, hepatitis C virus, hepatocellular carcinogenesis

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common cancers in the world. Until recently, hepatitis C virus (HCV) has been reported to be a causative agent of HCC aside from hepatitis B virus (HBV).[1–5] The annual incidence of HCC in patients with HCV RNA-positive cirrhosis ranges 5–7%.[5–7] The carcinogenesis rate was higher in those patients with cirrhosis caused by HCV than in those with HBV-related cirrhosis.[5]

Interferon (IFN) is effective in reducing HCC rate through suppression of necroinflammatory process, serum alanine aminotransferase (ALT) and in eliminating HCV in some patients with chronic HCV and cirrhosis. Although IFN proves to be valuable in suppression of the risk of carcinogenesis, it is not effective in every patient with HCV-related disease. Oka *et al.*[8] reported in a randomized controlled trial that a kind of medicinal herb, "Sho-saiko-to", could significantly decrease hepatic carcinogenesis rate in patients without hepatitis B surface antigen (HBsAg)-negative cirrhosis. Tarao *et al.*[9] showed that the HCC appearance rate was significantly higher in HCV-related cirrhotics with a high ALT value of 80 IU or more than that of those with lower ALT value, and also suggested that treatment of cirrhosis and prevention of HCC should be directed to suppress the necroinflammation of HCV-related hepatitis.

In Japan, a glycyrrhizin-containing herbal medicine, Stronger Neo-Minophagen C (SNMC), is widely used in Japan for the treatment of chronic hepatitis. It is used in the form of an i.v. solution, comprised of 0.2% glycyrrhizin, 0.1% cysteine and 0.2% glycine in physiological solution. It is made by dissolving glycyrrhizin (200 mg),

Correspondence: Dr Kenji Ikeda, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. Email: ikedakenji@tora.email.ne.jp

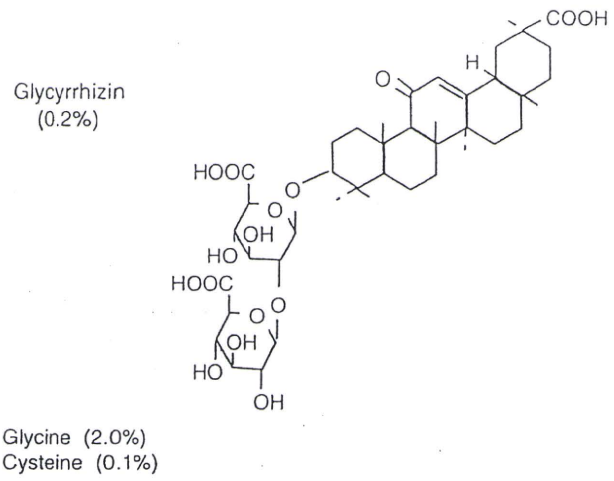

Figure 1 Chemical structure of glycyrrhizin and Stronger Neo-Minophagen C in physiological saline solution.

cysteine (100 mg), glycine (2 g) in 100 mL of physiological saline (Fig. 1). Glycyrrhizin is an aqueous extract of licorice root (*Glycyrrhizae radix*), which is anti-allergic and has detoxicating effects. As has been reported, the anti-inflammatory mechanism of glycyrrhizin is believed to be due to its protective effect on the hepatic cellular membrane, which may explain its ability to lower the serum transaminase level in patients with chronic hepatitis. Because glycyrrhizin has an anti-inflammatory action and favorable effect on ALT and histology in patients with chronic viral hepatitis,[10–15] we analyzed its effect on HCC in those patients with chronic HCV.[16]

In order 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 virological response.[17]

METHODS

Patients

A TOTAL OF 1249 consecutive Japanese patients with chronic hepatitis or cirrhosis type C were examined, who could not eradicate HCV RNA with previous IFN therapy. There were 778 men and 471 women aged 18–81 (median age, 53 years) in the study. They were diagnosed as having liver cirrhosis by peritoneoscopy, liver biopsy or both between 1987 and 2002 at Toranomon Hospital, Tokyo, Japan. All the patients had a history of receiving IFN therapy once or more. 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 showed abnormal ALT at 6 months after the end of IFN therapy.

Glycyrrhizin therapy

Of 1249 patients with IFN-resistant chronic liver disease, 453 patients underwent glycyrrhizin injection therapy (SNMC) and the remaining 796 patients did not receive glycyrrhizin therapy until the end of observation. The purpose for the introduction of glycyrrhizin therapy was to suppress high ALT levels and to prevent disease progression in all the patients. F1 stage hepatitis was significantly more often found in the untreated group than in the glycyrrhizin group ($P < 0.001$, χ^2 test). Both AST and ALT medians were significantly higher in the glycyrrhizin group than in the untreated group ($P < 0.001$).

When glycyrrhizin was regarded as effective from an aminotransferase viewpoint, treatment was usually continued for as long a period as possible. As a result, a median daily dose of 100 mL of glycyrrhizin was administered thrice weekly during a median period of 4.3 years (range, 0.1–14.5 years) in the treated group. Two (0.44%) of the 453 treated patients withdrew from glycyrrhizin injection therapy because of side-effects: one from hypertension and one from skin rash without itching.

Background and laboratory data of patients with and without 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 two groups. Median age was older by 2 years in the treated group than in the untreated group ($P < 0.001$). F1-stage hepatitis was found significantly more often in the untreated group than in the glycyrrhizin group ($P < 0.001$, χ^2 test). Median levels of both AST and ALT were significantly higher in the treated group than in the untreated group ($P < 0.001$). The rate of HCV serological group 1 was significantly higher in the glycyrrhizin group than in the untreated group ($P = 0.032$).

Follow up of the patients

Follow up of the patients was made on a monthly basis after the judgment of IFN resistance by monitoring hematological, biochemical, and virological data.

Table 1 Patients profiles and laboratory data at the time of judgment of interferon-resistance

	Glycyrrhizin group ($n = 453$)	Untreated group ($n = 796$)	*P*-value
Demography			
Sex (M/F)	283/170	495/301	0.92‡
Age (year)†	54 (25–81)	52 (18–77)	<0.001
Liver histology			
F1/F2/F3/F4	146/193/38/69	502/192/52/38	<0.001‡
Laboratory data†			
Aspartic transaminase (IU/L)†	81 (19–446)	54 (11–355)	<0.001
Alanine transaminase (IU/L)†	122 (12–630)	83 (10–822)	<0.001
HCV serological group 1/2	360/73	582/165	0.032‡

†Expressed by median (range). ‡χ^2 test or Mann–Whitney *U*-test.

Imaging diagnosis with ultrasonography (US) and/or computerized tomography (CT) was made three or more times per year in a majority of patients with cirrhosis, and once a year in patients without cirrhosis.

The numbers of cases lost to follow up were 121 (9.7%): 28 patients (6.2%) in the glycyrrhizin group and 93 (11.7%) in the untreated group. Because the eventual outcomes regarding appearance of HCC were not identified in these patients, they were dealt as censored data in the following statistics. 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.

Statistical analysis

Non-parametric 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 a period between the judgment of IFN ineffectiveness and the appearance of HCC in each group, using the Kaplan–Meier technique.[17] 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.[18] 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 a log-minus-log plot of a proportional hazard model.

All data analysis was performed using the computer program SPSS version 11 (SPSS, Chicago, IL, USA).

RESULTS

Initial aminotransferase and carcinogenesis rates

BECAUSE AMINOTRANSFERASE LEVEL is likely to affect future disease progression, entire patients of the cohort were classified into six categories according to average ALT value during the first year after cessation of IFN therapy: (i) normal ALT; (ii) less than 1.5 times of upper limit of normal (ULN); (ii) 1.5–2 times of ULN; (iv) 2–3 times of ULN; (v) 3–4 times of ULN; and (vi) more than 4 times of ULN. Hepatocellular carcinogenesis rates were 2.5%, 5.0%, 8.1%, 11.8%, 12.0% and 12.7% at the end of the fifth year, and 6.6%, 7.2%, 19.6%, 15.1%, 21.0% and 39.3% at the 10th year, respectively. There was a significant statistical difference among the six subgroups (log-rank test, $P < 0.0001$). The higher the average ALT, the higher the carcinogenesis rate was.

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 the untreated groups: (i) normal average ALT was found in 38 among patients with glycyrrhizin therapy and in 188 among patients without therapy; (ii) ALT of less than 1.5 times of ULN was found 42 and 331; (iii) 1.5 times to 2 times of ULN 84 and 138; (iv) 2–3 times of ULN in 143 and 92; (v) 3–4 times in 53 and 29; and (vi) ALT of more than 4 times of ULN in 93 of the glycyrrhizin group and 18 of the untreated group, respectively. 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).

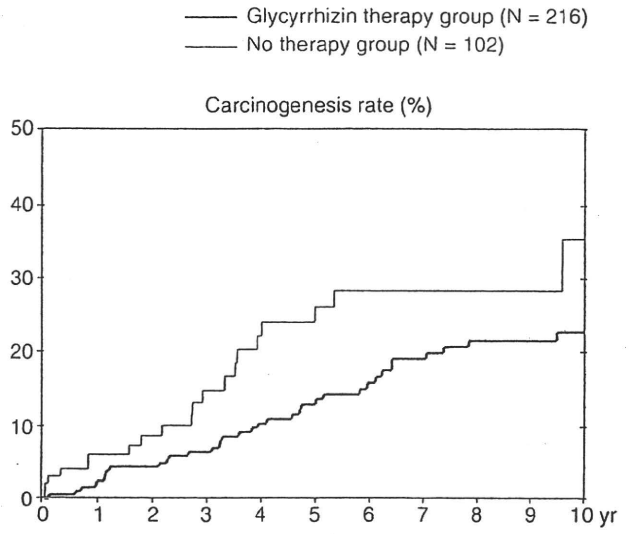

Figure 2 Carcinogenesis rates in patients with active chronic hepatitis showing high average alanine transferase (ALT) values of twice or more of upper limit of normal. Carcinogenesis rate in patients with a sufficient period of glycyrrhizin treatment was significantly lower than that of the untreated patients (log-rank test, $P = 0.021$).

Carcinogenesis in patients with high aminotransferase

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 on 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 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 the third year, 13.3% and 26.0% at the end of the fifth year, 17.7% and 28.3% at the end of the seventh year, and 21.5% and 35.5% at the 10th year, respectively (Fig. 2). In the stratified and selected patient group, the carcinogenesis rate of the glycyrrhizin-treated group was significantly lower than that of the untreated group (log-rank test, $P = 0.021$).

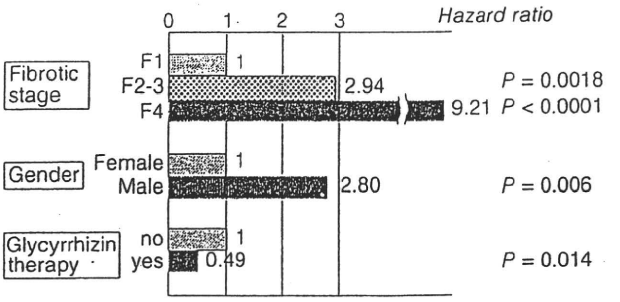

Figure 3 Independent risk factors affecting hepatocellular carcinogenesis (time-dependent Cox proportional hazard analysis).

Impact of glycyrrhizin therapy on carcinogenesis

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 a time-dependent Cox proportional hazard model. Time between the judgment of IFN ineffectiveness and initiation of glycyrrhizin therapy was set as a time-dependent variable, in order 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 in the analysis.

In multivariate analysis, the following three factors influenced the carcinogenesis: (i) fibrotic staging; (ii) sex ($P = 0.006$); and (iii) glycyrrhizin therapy ($P = 0.014$) (Fig. 3). When a hazard of F1-stage fibrosis for carcinogenesis was set as 1 in the model, the hazard ratio of F2 to F3 stage fibrosis was calculated as 2.94 ($P = 0.018$), and that of F4 (cirrhosis) was estimated as 9.21 ($P < 0.001$). Similarly, the hazard ratio for carcinogenesis of male gender was 2.80, compared to female. Use of glycyrrhizin independently decreased the carcinogenesis rate with a hazard ratio of 0.49, in patients with active chronic hepatitis after IFN therapy. The following factors did not affect the HCC appearance rate significantly: age, association of diabetes mellitus, serological grouping of HCV, HCV RNA concentration, AST, ALT at the time before IFN therapy, and bilirubin.

Carcinogenesis in elderly patients

Cumulative carcinogenesis rates were compared between patients with and without glycyrrhizin therapy, in a subgroup of older patients of 60 years old or more. Carcinogenesis rates in the treated ($n = 58$) and

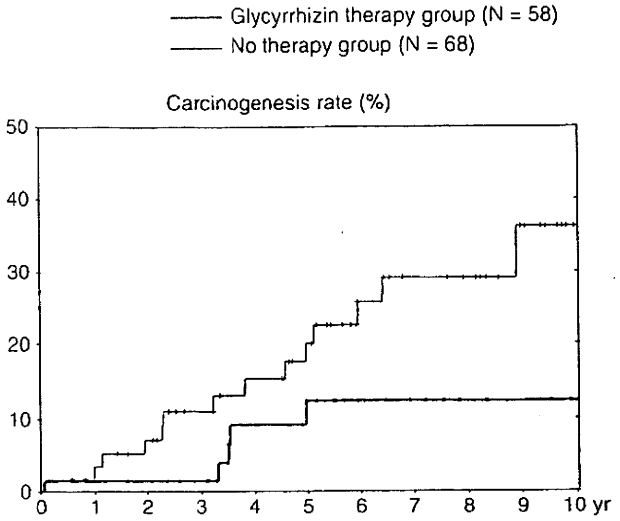

Figure 4 Carcinogenesis rates in elderly patients of 60 years or more.

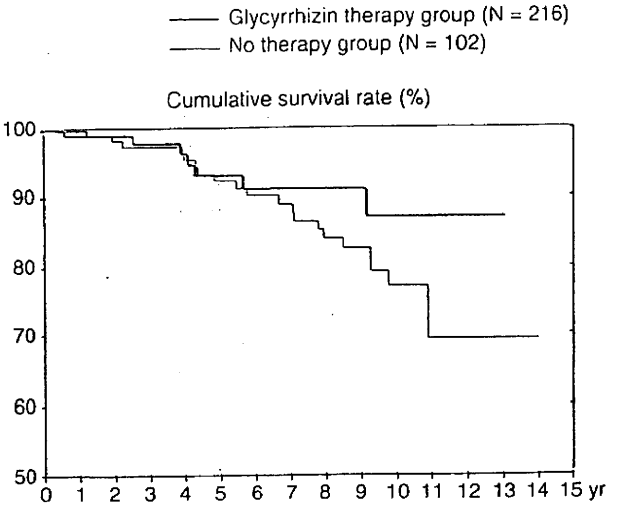

Figure 5 Crude survival rates in patients with and without glycyrrhizin treatment.

untreated groups ($n = 68$) were 12.4% and 20.0% at the end of the fifth year, and 12.4% and 36.2% at the 10th year, respectively (Fig. 4). The carcinogenesis rate in the glycyrrhizin injection group apparently decreased, but marginal statistical difference was observed (log-rank test, $P = 0.052$).

Survival rate

Cumulative survival rates after cessation of IFN therapy were calculated in the treated and untreated groups. Five-year survival rates in patients with and without glycyrrhizin injection therapy were 93.3% and 92.5%, and 10-year rates were 87.2% and 77.1%, respectively (Fig. 5). Although statistical significance was not obtained in the survival rates between the two groups, it showed higher rates in the treated group than in the untreated group.

DISCUSSION

YAMAMOTO *ET AL.*[10] first treated patients with chronic hepatitis with glycyrrhizin (SNMC) and found a distinct improvement in their ALT levels. Suzuki *et al.*[12] confirmed its ability to suppress serum aminotransferase in patients with chronic hepatitis in a randomized controlled trial. Hino *et al.*[13] and Yasuda *et al.*[14] also proved glycyrrhizin to be useful in the improvement of transaminase and liver histology. We once reported that glycyrrhizin was beneficial in carcinogenesis rate in patients with chronic hepatitis type C when it was administered for 10 years or longer.[19] In this study,[20] we assessed the role of glycyrrhizin in the prevention of hepatocellular carcinogenesis in patients with INF-resistant chronic hepatitis C.

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 actually difficult from both ethical and medical viewpoints in Japan, where glycyrrhizin injection therapy is covered by standard medical insurance and it is already regarded as a usual salvaging procedure for IFN-ineffective patients. Therefore, we attempted to carry out a retrospective cohort study, with a statistical adjustment using possible covariates explored in multivariate analysis.

When crude carcinogenesis rates were compared between the treated and untreated patient group, the hepatocellular carcinogenesis rate in the glycyrrhizin therapy group was higher than that of the untreated group (data not shown). Because anti-inflammatory therapy using glycyrrhizin was usually performed for those patients with high ALT values and more active hepatitis, it seemed a quite convincing result that the carcinogenesis rate of the treated group was higher than that of the untreated group. Actually, the treated group consisted of significantly more numbers of patients with high ALT values of twice or more of ULN. When carcinogenesis rates were assessed only in patients with high ALT values of twice or more ULN, the rate of the treated group became slightly higher than that of the

untreated group. Of patients in the treated group, some of them received glycyrrhizin injection therapy several months or a few years after judgment of IFN ineffectiveness. In order to elucidate the cancer preventive activity of glycyrrhizin in active HCV-related liver disease, we further stratified the treated patients into two groups: (i) early treatment group of glycyrrhizin within 2 years after judgment of IFN ineffectiveness; and (ii) late treatment group after 2 years. Because the latter patients were observed without therapy for a considerable period inspite of the "treated group", they were regarded as partly and insufficiently treated with glycyrrhizin from a viewpoint of the entire observation period. We therefore compared the carcinogenesis rates between the treated and untreated patients, excluding those patients of a late treatment group.

The hepatocellular carcinogenesis rate of the patients with a sufficient period of glycyrrhizin injection was significantly lower than that of those without therapy ($P = 0.038$). In the treated group, median ALT values significantly decreased after initiation of the glycyrrhizin injection, suggesting that suppression of the necroinflammatory process was the principal mechanism of the anti-carcinogenic activity of the medicine. The current study dealing with a large cohort ($n = 1249$), showed that the carcinogenesis rate reduces when glycyrrhizin therapy is started at an early time after judgment of IFN ineffectiveness. Cancer preventive activity of glycyrrhizin was also found in a subgroup of elderly patients 60 years or older. Because glycyrrhizin therapy has few side-effects, it should be taken into account for the treatment of aged patients with chronic hepatitis C, from the viewpoint of cancer prevention. Survival rate is likely to increase in those patients undergoing long-term glycyrrhizin injection therapy through suppression of aggressive necroinflammatory process and suppression of liver-related morbidity and mortality.

CONCLUSIONS

AS CARCINOGENESIS IS not a single-step event, but a complex, multistep process, the exact mechanism of the glycyrrhizin activity in suppression of liver carcinogenesis still remains unknown. One of the principal roles of long-term administration of glycyrrhizin in decreasing the carcinogenesis rate seemed to be anti-inflammatory ones, which would retrieve an active carcinogenic process with ALT elevation and continuous hepatic necroinflammation. Glycyrrhizin may only postpone the time of HCC appearance in the clinical course of cirrhosis. Because the entire process of hepatocellular carcinogenesis from initial transformation of a hepatocyte to detectable growth is considered to take at least a few years, the influence of glycyrrhizin on the carcinogenesis rate will not be evaluated in a short period of a few years. Future studies should therefore be aimed 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.

CONFLICT OF INTEREST

NO CONFLICT OF interest statement has been received from the author.

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Long-Term Outcome after Interferon Therapy in Elderly Patients with Chronic Hepatitis C

Yasuji Arase Kenji Ikeda Fumitaka Suzuki Yoshiyuki Suzuki Satoshi Saitoh
Masahiro Kobayashi Norio Akuta Takashi Someya Rikako Koyama
Tetsuya Hosaka Hitomi Sezaki Mariko Kobayashi Hiromitsu Kumada

Department of Gastroenterology, and Hepatic Research Unit, Toranomon Hospital, Toranomon, Tokyo, Japan

Key Words
Chronic hepatitis C · Elderly patients · Interferon · Hepatocellular carcinoma · IFN therapy in elderly patients, survival

Abstract
Objective: The purpose of this study was to elucidate the long-term outcome after interferon (IFN) therapy in chronic hepatitis C elderly patients. ***Methods:*** We studied the incidence of hepatocellular carcinoma (HCC) and survival probability after the initiation of IFN therapy in 500 Japanese chronic hepatitis C patients >60 years. The mean age of initiation of IFN was 63 years and the mean follow-up period was 7.4 years. Cox proportional hazard regression analysis was used to evaluate the long-term outcome after initiation of IFN therapy. Sustained virological response (SVR) was defined as negative HCV-RNA by RT-nested PCR 6 months after the completion of long-term IFN therapy. Non-response (NR) was applied to patients who did not show SVR. Hepatic fibrosis was defined as the fibrosis score (score 0–4) according to Knodell et al. ***Results:*** 140 patients (28%) had an SVR and 360 patients (72%) had an NR. 71 of 500 patients developed HCC during follow-up. The cumulative incidence of HCC was 9.6% at the 5th year, 17.4% at the 10th year, and 31.3% at the 15th year. HCC developed with significance when: (1) HCV was not cleared after IFN therapy ($p < 0.0001$), (2) sex was male ($p < 0.0001$), and (3) staging of liver fibrosis was >2 ($p = 0.008$). 53 of the patients died. The cumulative survival probability was 95.7% at the 5th year, 86.4% at the 10th year, and 78% at the 15th year. Patients achieved a long survival with significance when: (1) staging of liver fibrosis was 1 ($p < 0.0001$), (2) HCV was cleared after IFN therapy ($p = 0.034$), and (3) sex was female ($p = 0.015$). ***Conclusion:*** Chronic hepatitis C patients with clearance of HCV after IFN therapy had a significantly reduced risk of HCC appearance and achieved prolonged survival even if they are ≥60 years.

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 per year is attributed to the persistent infection with HCV in Japan [2, 3]. It is important to eradicate HCV or decrease levels of

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Yasuji Arase, MD
Department of Gastroenterology, Toranomon Hospital
2-2-2 Toranomon, Minato-ku, Tokyo 105-8470 (Japan)
Tel. +81 3 3588 1111, Fax +81 3 3582 7068
E-Mail es9y-ars@asahi-net.or.jp

alanine aminotransferase (ALT) for preventing HCC with interferon (IFN) therapy [4, 5].

Nowadays, patients with HCV in Japan tend to be aged. Also, HCV-related HCC patients have been shown to become old with a peak around the age of 70 [6]. When such aged chronic hepatitis C patients with abnormal ALT levels consult a doctor, the first problem is whether or not therapy should be used for chronic hepatitis C. Moreover, when treatment for chronic hepatitis C is decided in such aged patients, whether IFN therapy should be used or not is the second problem. However, a few studies have targeted IFN therapy and prolonged prognosis in elderly patients with chronic hepatitis C [7, 8]. Until now, IFN treatment for chronic hepatitis C has mainly been introduced when patients are less than 60–65 years of age because of IFN-related side effects and safety standards in Japan. Owing to IFN-related side effects or various complicated diseases, there is a tendency not to give IFN to aged patients. Thus, IFN therapy for chronic hepatitis C has been conventionally limited to patients aged less than 60–65 years. We therefore assessed the long-term efficacy of IFN therapy in elderly patients with chronic hepatitis C by a retrospective cohort study.

Patients and Methods

Patients

The number of chronic hepatitis C patients treated with IFN therapy in our hospital between 1989 and 2004 was 3,320. Of these, 500 patients had the following criteria: (1) ≥ 60 years of age; (2) ALT elevation greater than double the upper limits (ALT normal range 12–50 IU/l) within 6 months; (3) no corticosteroid immunosuppressive agents, or antiviral agents used within 6 months; (4) no hepatitis B surface antigens, antinuclear antibodies, or antimitochondrial antibodies detectable in serum, determined by radioimmunoassay; (5) leukocytes >3,000/mm^3, platelet count >80,000/mm^3, and bilirubin <2.0 mg/ml, and (6) IFN therapy >4 weeks. Next, we excluded those patients from the study with a history of alcohol abuse or advanced liver cirrhosis of encephalopathy, bleeding esophageal varices, or ascites. 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 the potential adverse reactions to each patient, who later gave his/her informed consent for participation.

IFN Therapy

For the first IFN treatment regimen, the IFN treatment consisted of 3–12 million units (MU) of IFN-α or IFN-β. For the IFN treatment regimen, one group of 245 patients was assigned to receive IFN intramuscularly every day for the first 2–8 weeks and then 2–3 times/week for the following 16–96 weeks. Another group of 116 cases was assigned to receive IFN 3 times/week for 24–104 weeks. A third group of 108 patients was assigned to be treated with IFN by intravenous injection daily for 4–8 weeks. The fourth group of 31 patients was given combination therapy of IFN and ribavirin. The median total dose was 624 MU (range 168–2,430) and median administration period was 165 days (range 28–730).

Definition of Response of IFN Efficacy

Patients treated with IFN were divided into the following two groups based on the serum HCV-RNA after the termination of IFN. Sustained virological response (SVR) was defined as negative HCV-RNA by RT-nested PCR 6 months after the completion of long-term IFN therapy. Non-response (NR) was applied to patients who did not show SVR.

Blood and Urine Tests

Blood samples were obtained just before and 6 months after IFN treatment. The samples were stored at –80° until analyzed. Using these blood samples, HCV-RNA levels before IFN therapy were analyzed by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems) [9]. On the other hand, HCV-RNA 6 months after the termination of IFN therapy was analyzed by qualitative PCR assay. The lower detection limit of the qualitative assay is 100 copies/ml [10]. HCV genotype was examined by PCR assay, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [11].

Follow-Up Protocol

The start of the follow-up period was defined as the first day of IFN treatment. Clinical evaluation and biochemical and hematologic tests were performed at 1–3 monthly intervals. Thirty-four patients were lost to follow-up. Because the appearance of HCC and death was not identified in these 34 patients, they were considered as censored data in statistical analyses [12]. Moreover, patients retreated with IFN in order to eradicate HCV-RNA were regarded as withdrawals at the start of IFN retreatment.

Diagnosis of HCC was based on the presence of typical hypervascular characteristics on angiography, in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy material was performed in patients whose angiograms did not demonstrate a typical image of HCC. Histopathological confirmation using surgically resected specimens was made in 21 patients.

Cause of death was divided into liver-related and liver-unrelated. The former included HCC, liver failure, and esophagogastric variceal bleeding, and the latter included extrahepatic malignancies, heart disease, cerebrovascular accidents, pulmonary disease, and others.

Liver Histology before IFN Therapy

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The biopsy specimens were scored according to the system of Knodell et al. [13]. Histologic index score: 0–10 for periportal bridging necrosis and 0–4 for interlobular degeneration and focal necrosis, portal inflammation, and fibrosis.

Table 1. Clinical characteristics before IFN treatment according to efficacy of IFN therapy in chronic hepatitis C elderly patients

Characteristics	SVR (n = 140)	NR (n = 360)	p
Age, years*	63 ± 3.2	64 ± 3.2	0.070
Male/female	83/57	168/192	0.011
Liver histology (fibrosis: 1/2/3/4)**	59/47/6/12	120/109/29/58	0.009
Liver histology (activity)*, **	9.3 ± 3.4	9.8 ± 2.7	0.223
HCV genotype (1b/2a/2b/others)	47/74/12/17	255/48/38/23	<0.0001
HCV load, kIU/ml*	172 ± 204	661 ± 506	<0.0001
AST, IU/l*	83 ± 70	87 ± 51	0.143
ALT, IU/l*	113 ± 102	118 ± 82	0.200
Hb, g/dl	14.2 ± 1.4	14.1 ± 1.3	0.547
Platelets, $\times 10^4/mm^3$*	15.0 ± 4.6	14.9 ± 4.8	0.768
WBC, $\times 10^3/mm^3$*	4.6 ± 1.4	4.6 ± 1.3	0.751
Period of observation, years	7.0 ± 3.3	7.7 ± 3.6	0.011

Activity was defined as sum score of periportal bridging necrosis, interlobular degeneration and focal necrosis, and portal inflammation.

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; NR = non-response; SVR = sustained virological response; WBC = white blood cells.

* Data are number of patients or mean ± SD.

** Histologic index score: 0–10 for periportal bridging necrosis and 0–4 for interlobular degeneration and focal necrosis, portal inflammation, and fibrosis.

Activity was defined as sum score of periportal bridging necrosis (score 0–10), interlobular degeneration and focal necrosis (score 0–4), and portal inflammation (score 0–4). Fibrosis was defined as fibrosis score (score 0–4).

Statistical Analysis

Baseline characteristics and treatment differences among groups based on efficacy of IFN treatment were analyzed using Kruskal-Wallis test. HCC appearance rates were analyzed by the log-rank test. A Cox proportional hazards model was used to analyze the factors contributing to the HCC appearance rate and death: factors examined included age, gender, histologic findings, HCV genotype, HCV load, aspartate aminotransferase (AST), ALT, and efficacy of IFN administration. A p value <0.05 was considered statistically significant. The SPSS Software Package (SPSS Inc., Chicago, Ill., USA) was used for analyses.

Results

Characteristics of the Patients and the Efficacy of the IFN Therapy

500 patients were enrolled in the present study. 140 patients (28%) had a SVR and 360 patients (72%) had a NR. Table 1 shows the baseline characteristics of the patients based on the efficacy of IFN therapy. The frequency distributions of the HCV genotype, the stage of liver fibrosis and HCV load differed between the two groups.

Development of HCC and Risk Factors for Appearance of HCC

During follow-up, HCC developed in 71 patients. The cumulative incidence as shown in figure 1 was based on efficacy of IFN therapy. The cumulative incidence of HCC was 9.6% at the 5th year, 17.4% at 10th year, and 31.3% at 15th year.

Cox regression analysis was performed using nine variables, including age, sex, histopathological severity (staging), viral load, HCV genotype, serum AST, serum ALT, and efficacy of IFN therapy. Univariate analysis showed that the following five factors significantly affected the cumulative HCC appearance rate in all patients as shown in table 2. Because the variables were mutually correlated, multivariate Cox regression analysis was performed with the statistically significant variables in the model (table 3). HCC developed with significance when: (1) HCV was not cleared ($p < 0.0001$), (2) sex was male ($p < 0.0001$), and (3) staging of liver fibrosis was >2 ($p = 0.008$).

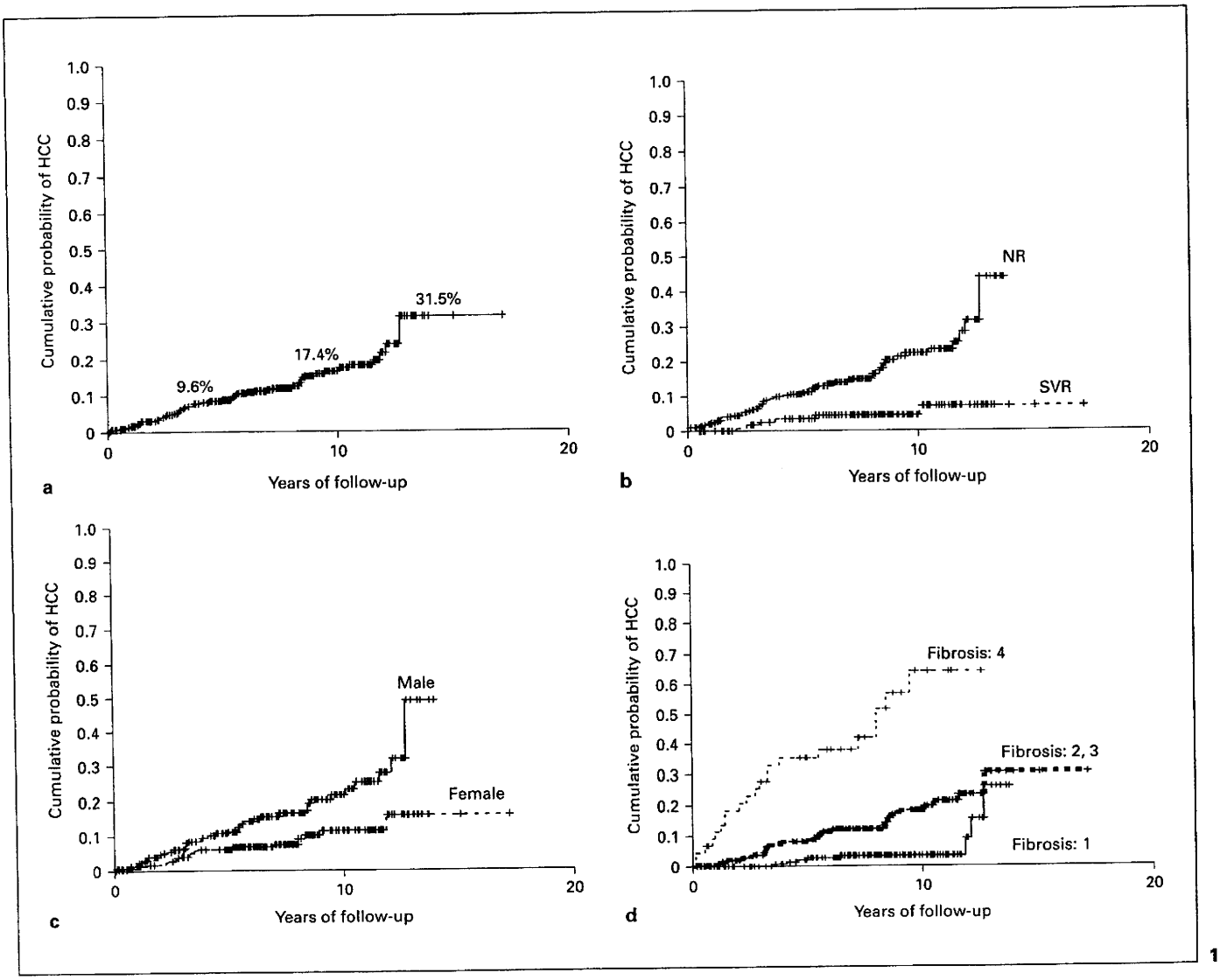
Fig. 1. Cumulative appearance probability of HCC. **a** In total patients; **b** based on difference of efficacy of IFN therapy; **c** based on difference of sex; **d** based on difference of histological fibrosis. SVR = Sustained virological response; NR = non-response.

Table 2. Predictive factors for hepatocellular carcinoma appearance after IFN therapy by Cox proportional hazards model (univariate analysis)

Factor	Category	Risk ratio*	95% CI	p
Liver histology (fibrosis)	1/2,3,4	1/4.22	2.81–6.34	<0.0001
Sex	Male/female	1/0.44	0.26–0.75	0.002
Age, years	<65/≥65	1/1.95	1.14–3.32	0.015
HCV genotype	1/2	1/0.55	0.31–0.97	0.046
AST, IU/l	<76/≥76	1/1.75	0.83–3.67	0.141
ALT, IU/l	<100/≥100	1/1.64	0.79–3.40	0.184
HCV-RNA, kIU/ml	<100/≥100	1/1.47	0.79–2.76	0.224
Liver histology (activity)	<10/≥10	1/1.55	0.79–3.07	0.206
Efficacy of IFN therapy	NR/SVR	1/0.22	0.096–0.52	<0.0001

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; NR = non-response; SVR = sustained virological response.

* Risk ratio for development of HCC (71 events among all 500 patients) were calculated by using Cox proportional hazards regression analysis.



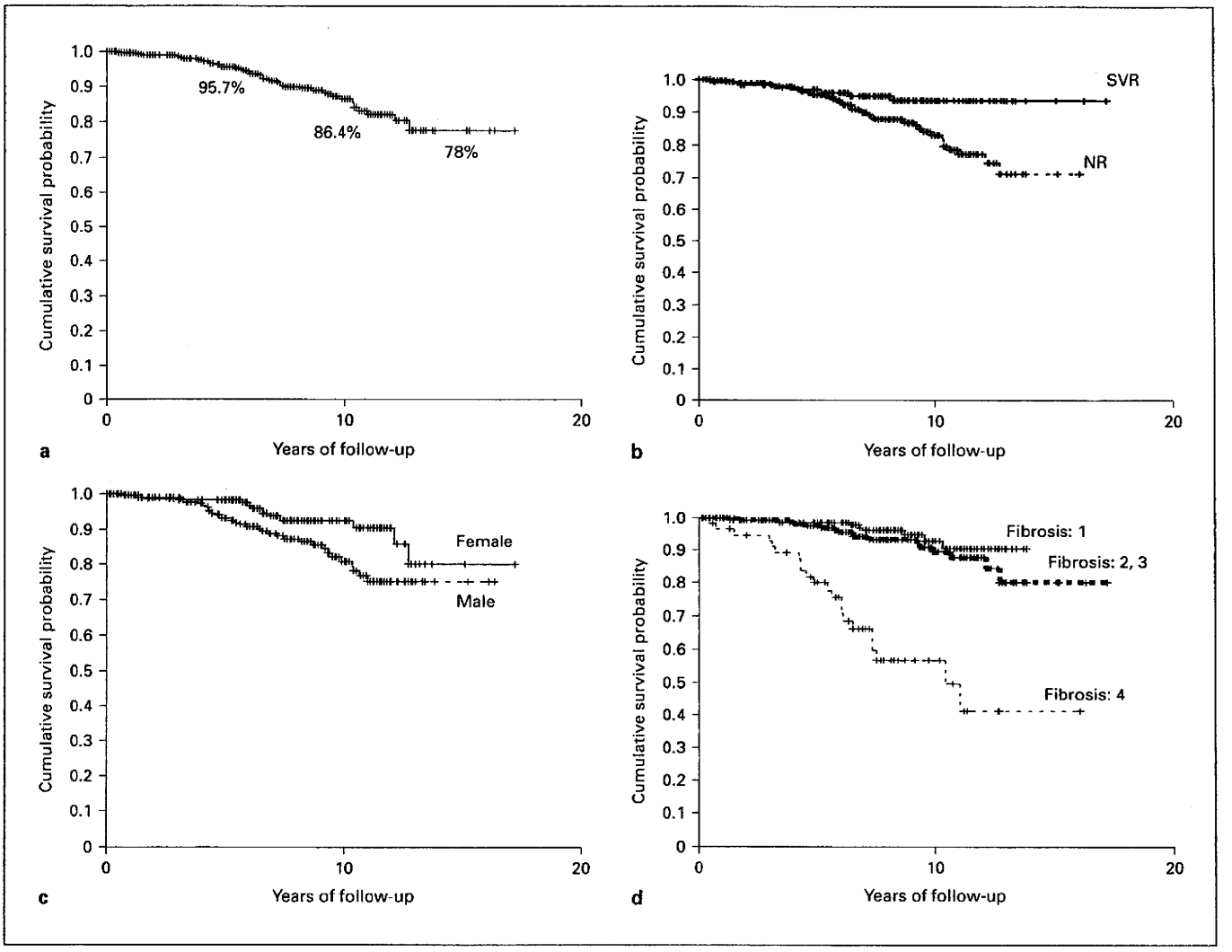


Fig. 2. Cumulative survival probability after IFN therapy. **a** In all patients; **b** based on difference of efficacy of IFN therapy; **c** based on difference of sex; **d** based on difference of efficacy of histological fibrosis. SVR = Sustained virological response; NR = non-response.

Table 3. Predictive factors for hepatocellular carcinoma appearance after IFN therapy by Cox proportional hazards model (multivariate analysis)

Factor	Category	Risk ratio	95% CI	p
Efficacy of IFN therapy	NR/SVR	1/0.193	0.083–0.45	<0.0001
Sex	Male/female	1/0.36	0.21–0.62	<0.0001
Liver histology (fibrosis)*	1/2,3,4	1/2.08	1.22–3.57	0.008

ALT = Alanine aminotransferase; CI = confidence interval; NR = non-response; SVR = sustained virological response.

* Histologic index score: 0–4 for fibrosis.

Cause of Death and Cumulative Survival Probability

Fifty-three of 500 patients died during an average follow-up of 7.4 years. The cumulative survival probability was 95.7% at the 5th year, 86.4% at the 10th year, and 78% at the 15th year (fig. 2). The number of liver-related and liver-unrelated deaths is shown in table 4. Liver-related death corresponded to 64.2% (34/53) of all deaths. HCC was the major cause of liver-related deaths. Univariate analysis showed that the following four factors significantly affected the cumulative survival probability in all patients as shown in table 5. Multivariate analysis revealed that patients achieved a significant survival when: (1) staging of liver fibrosis was 1 ($p < 0.0001$), (2) HCV was cleared ($p = 0.034$), and (3) sex was female ($p = 0.015$).

Table 4. Cause of death

	Efficacy of IFN	
	SVR (n = 140)	NR (n = 360)
Deaths	9	44
Liver related	2 (22%)	32 (73%)
Hepatocellular carcinoma	2	26
Liver failure	0	5
Gastrointestinal bleeding	0	1
Liver unrelated	7 (78%)	12 (27%)
Malignancies	3	7
Heart disease	2	2
Cerebrovascular disease	2	1
Pulmonary disease	1	2

SVR = Sustained virological response; NR = non-response.

Discussion

In order to protect hepatocarcinogenesis, patients with chronic hepatitis C are often treated with antivirus drugs and/or anti-inflammatory drugs. Antivirus drugs, such as IFN, are given to eradicate HCV-RNA for chronic hepatitis C. Moreover, it has recently been reported that hepatitis C viral clearance or normalization of serum ALT after IFN therapy contribute to the notably suppressed incidence of HCC caused by chronic HCV infection [14–17]. Yoshida et al. [14] reported the efficacy of IFN for HCV-positive patients with chronic hepatitis or cirrhosis in a retrospective surveillance study in Japan, which focused on the HCC appearance rate and survival in a total of 2,890 patients, 2,400 treated with IFN and 490 not treated with IFN. Their conclusion was that IFN therapy improved survival of chronic hepatitis C patients by preventing liver-related deaths. Ikeda et al. [15] reported similar results from 1,643 patients of whom 1,191 had received IFN monotherapy. The incidence of HCC in treated patients was 7.6% after 10 years of follow-up evaluation, compared with 12.4% in untreated patients. Imazeki et al. [16] presented a follow-up study of 459 patients with chronic hepatitis C over 8.2 years to examine the survival rate relative to IFN therapy. Cirrhotic patients with a SVR showed a reduction in mortality (relative risk 0.219). Serfaty et al. [17] showed a beneficial effect of IFN on the development of HCC and on survival.

In the present study we found that the clearance of HCV after IFN therapy reduced HCC appearance and liver-related death in elderly patients (table 6). Horiike et al. [7] reported that elderly patients with a low HCV-RNA

Table 5. Predictive factors for survival probability after IFN therapy by Cox proportional hazards model (univariate analysis)

Factor	Category	Risk ratio*	95% CI	p
Liver histology (fibrosis)	1/2,3/4	1/3.90	2.49–6.10	<0.0001
Sex	Male/female	1/0.47	0.26–0.86	0.014
Efficacy of IFN therapy	NR/SVR	1/0.37	0.17–0.83	0.015
Age, years	<65/≥65	1/2.08	1.14–3.78	0.016
HCV genotype	1/2	1/0.55	0.31–0.97	0.046
AST, IU/l	<76/≥76	1/0.80	0.37–1.74	0.571
ALT, IU/l	<100/≥100	1/0.81	0.37–1.76	0.588
HCV-RNA, kIU/ml	<100/≥100	1/0.92	0.49–1.71	0.781
Liver histology (activity)	<10/≥10	1/1.62	0.73–3.62	0.206

ALT = Alanine aminotransferase; AST = aspartate aminotransferase; CI = confidence interval; NR = non-response; SVR = sustained virological response.

* Risk ratio for development of HCC (71 events among all 500 patients) were calculated by using Cox proportional hazards regression analysis.

Table 6. Predictive factors for death after IFN therapy

Factors (category)	Overall deaths		Liver-related death		Liver-unrelated death	
	risk ratio* (95% CI)	p	risk ratio* (95% CI)	p	risk ratio* (95% CI)	p
Liver histology (fibrosis: 1/2,3,4)	1/3.55 (2.28–5.51)	<0.0001	1/6.18 (3.37–11.35)	<0.0001	1/1.10 (0.52–2.36)	0.799
Efficacy after IFN therapy (NR/SVR)	1/0.39 (0.16–0.93)	0.034	1/0.13 (0.03–0.59)	0.007	1/0.79 (0.27–2.32)	0.668
Male/female	1/0.46 (0.25–0.86)	0.015	1/0.40 (0.18–0.88)	0.022	1/0.71 (0.25–2.00)	0.799

CI = Confidence interval; IFN = interferon; NR = non-response; SVR = sustained virological response.
* Risk ratio for development of death (53 events among all 500 patients) were calculated by using Cox proportional hazards regression analysis.

level might benefit from IFN therapy, although they should decide the indications for IFN very carefully in this age group. Imai et al. [8] reported that some aged patients with chronic hepatitis C might be recommended IFN therapy.

Next, IFN therapy can be associated with various side effects and is costly, therefore the selection of aged patients for IFN therapy is extremely important. Factors predictive of a SVR to IFN have been extensively studied, i.e. short duration of disease, young age, absence of cirrhosis, low HCV-RNA levels and HCV genotype 2a [18]. Chronic hepatitis C patients with genotype 2a/2b or genotype 1b and lower virus load show a particularly good response to IFN therapy. Even if small amounts of IFN are given to patients with these factors, a biochemical response as well as a SVR can be expected. Therefore, when aged patients with chronic hepatitis C have various factors that would respond well to IFN treatment, IFN therapy can be recommended to aim for prolonged survival after screening for diseases other than chronic hepatitis C. On the other hand, when aged patients with chronic hepatitis C do not have factors that would respond well to IFN treatment, IFN therapy should not be recommended.

In conclusion, our results suggest that clearance of HCV after IFN therapy significantly reduces the risk of HCC appearance and death in aged chronic hepatitis C patients.

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Prolonged-Interferon Therapy Reduces Hepatocarcinogenesis in Aged-Patients With Chronic Hepatitis C

Yasuji Arase,[1]* Kenji Ikeda,[1] Fumitaka Suzuki,[1] Yoshiyuki Suzuki,[1] Masahiro Kobayashi,[1] Norio Akuta,[1] Tetsuya Hosaka,[1] Hitomi Sezaki,[1] Hiromi Yatsuji,[1] Yusuke Kawamura,[1] Mariko Kobayashi,[2] and Hiromitsu Kumada[1]

[1]*Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo, Japan*
[2]*Hepatic Research Unit, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo, Japan*

The aim of this study was to elucidate the reduction of hepatocarcinogenesis by prolonged interferon (IFN) monotherapy in aged chronic hepatitis C patients. Inclusion criteria were biopsy-proven chronic hepatitis or liver cirrhosis, 60 years and over, elevated serum aminotransferase and positive hepatitis C virus (HCV)-RNA. One hundred and twenty patients satisfied the above criteria were treated with natural IFN-alpha (dose: 3 million unit (MU), two or three times weekly for 0.5–15.5 years, mean 2.47 years) (IFN group). Another 240 patients treated with herbal medicines excluding IFN were selected as control (no-IFN group). The patients not treated with IFN were matched 2:1 with IFN group patients for sex and age. The clinical and biological differences were compared after treatment with the IFN group and the untreated group. Serum alpha-fetoprotein (AFP) level decreased with statistical significance after initiation of treatment with IFN compared to no treatment. The 5- and 10-year cumulative rates of hepatocellular carcinoma (HCC) were 5.9 and 13.7%, and 17.1 and 32.8%, for the IFN and untreated group, respectively. HCC development occurred when histologic staging was advanced, and IFN was not given, the AFP level after treatment was >10 ng/ml. Cox regression analysis indicated that the relative risk of HCC in patients in the IFN group was 0.3 times of that in the untreated patients. The relative risk rate for HCC in severe fibrosis was 3.9 compared with mild or moderate fibrosis. In conclusion, long-term IFN therapy for aged patients with chronic HCV infection is effective in decreasing the serum AFP level and preventing hepatocarcinogenesis. ***J. Med. Virol. 79:1095–1102, 2007.***

KEY WORDS: chronic hepatitis C; hepatocellular carcinoma; long-term interferon treatment; alpha-fetoprotein

INTRODUCTION

Current interferon (IFN) therapy for patients with chronic hepatitis C viral infection has been directed at viral clearance. Recent studies described improvement of therapeutic efficacy when IFN was combined with ribavirin [Schalm et al., 1997; McHutchison et al., 1998; Poynard et al., 1998; Reichard et al., 1998]. Novel long-acting formulations of IFN known as pegylated IFN induced a higher eradication rate of hepatitis C virus (HCV) [Zuzem et al., 2000; Lindsay et al., 2001; Manns et al., 2001]. However, some patients do not clear the virus despite these new IFN therapies. Failure of HCV clearance could lead potentially to liver cirrhosis and/or hepatocellular carcinoma (HCC) [Imai et al., 1998; Yoshida et al., 1999]. Some patients cannot be given full doses of IFN because of IFN-related side effects. Thus, it is necessary to develop a new strategy for preventing the development of HCC in patients who cannot clear HCV-RNA regardless of IFN therapy and cannot be given full doses of IFN because of related side effects.

IFN can prevent the development of some malignancies apart from eradicating HCV [Gutterman, 1994;

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon.

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*Correspondence to: Yasuji Arase, MD, Department of Hepatology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan. E-mail: es9y-ars@asahi-net.or.jp

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Fogler et al., 1994; Scarpa et al., 1997; Murphy et al., 2001; Lindner, 2002]. A few previous studies indicate that long-term IFN therapy reduces the development of HCC in patients with HCV and chronic hepatitis or cirrhosis [Nishiguchi et al., 1995; Ikeda et al., 2000]. However, there is also controversy as to whether patients should be treated to prevent the development of HCC.

Some patients in Japan with chronic hepatitis C were, generally, aged. Also, HCV-related HCC patients have been shown to become old with a peak around age 70. Thus, this match-controlled study was conducted to evaluate the effect of long-term IFN therapy on the development of HCC in aged patients with HCV and with chronic hepatitis or cirrhosis.

MATERIALS AND METHODS

Patients

The number of patients who were diagnosed with chronic HCV infection and were subsequently treated with IFN monotherapy or IFN and ribavirin combination therapy between April 1991 and March 2006 was 4,250. Seven hundred and twenty of these patients had the following criteria: (1) laparoscopy and liver biopsy which showed histopathological features of chronic hepatitis or cirrhosis was taken within 1 year of initiation of IFN therapy; (2) 60 years and over; (3) positive for HCV-RNA by the amplicor monitor assay [Albadalejo et al., 1998] or reverse transcription nested polymerase chain reaction (RT-nested PCR) [Hagiwara et al., 1992]; (4) average alanine aminotransferase (ALT) elevation greater than 1.5 times the upper normal limits (ALT normal range: 12–50 IU) for more than 6 months before IFN therapy; (5) no treatment with corticosteroids, immunosuppressive agents, or antiviral agents within 12 months; (6) negative for hepatitis B surface antigens (HBsAg), antinuclear antibodies (ANA), or antimitochondrial antibodies (AMA) in the serum, as determined by radioimmunoassay and spot hybridization; (7) Leukocytes $>2{,}500/mm^3$, platelets $>70{,}000/mm^3$, and bilirubin <2.0 mg/ml before the initial period of IFN therapy; and (8) no evidence of HCC nodules by ultrasonography and/or computerized tomography within 1 month before IFN therapy.

Of the 720 patients satisfied with above criteria, 120 received IFN to prevent the development of HCC at a dose of 3 million units (MU) of natural IFN-alpha (Sumitomo Pharmaceutical Co., Osaka, Japan) two or three times a week for 2.47 ± 2.65 years. The decision for IFN therapy was made mainly after discussion between physician and patient. The patients were prospectively monitored the serum aminotransferase, alpha-fetoprotein (AFP), and HCC development. On the other hands, out of 720 patients with the above criteria, 240 patients (no-IFN group) treated without IFN were selected retrospectively so that no-IFN group patients were matched 2:1 with IFN group patients for sex, ages, and severe fibrosis. Patients with either of the following criteria were excluded from the study: (1) AFP of 400 ng/ml or higher, (2) advanced and decompensated stage of cirrhosis with encephalopathy, icterus, or refractory ascites, (3) a short follow-up period of 6 months or less, or (4) IFN was given daily, at a dose of >6 MU or other IFN excluded natural IFN-alpha. We compared the clinical and biological differences between IFN group and untreated group. The patients not treated with IFN were given herbal medicines (e.g., vitamin K, ursodeoxycholic acid, glycyrrhizin) [Takano et al., 1994; Arase et al., 1997; Tsubota et al., 1999]. Some of these substances improve serum transaminase and/or protecting HCC appearance [Takano et al., 1994; Arase et al., 1997; Tsubota et al., 1999]. Therefore, these drugs have been used for chronic hepatitis or cirrhosis in Japan since 1979. Untreated patient did not receive corticosteroids, immunosuppressive agents, or antiviral agents during the first stage of treatment. The study was approved by the institutional ethics review. Each patient gave informed consent.

Blood Tests

Blood samples were obtained just before treatment and stored at −80°C. HCV-RNA levels before therapy were treated by quantitative PCR assay (Amplicor GT-HCV Monitor Version 2.0, Roche Molecular Systems, CA) [Doglio et al., 1999]. Serum HCV-RNA every 2 or 3 month after the initiation of therapy in IFN group was examined by the qualitative PCR assay or RT nested PCR. The lower detection limit of the qualitative assay is 100 copies/ml. HCV genotype was examined by PCR, using a mixture of primers for the six subtypes known to exist in Japan, as reported previously [Dusheiko et al., 1994].

Follow-Up Protocol

Follow-up began on the first day of IFN treatment. In control group, follow-up began on the first day of herbal medicines. Clinical evaluation and biochemical and hematological tests were undertaken at monthly intervals. Twenty-one patients were lost to follow-up. Because HCC did not develop in these 21 patients, they were removed from the subject of this study at the time of final consultation in statistical analysis [Harrington and Fleming, 1983]. Deaths unrelated to HCC and patients who started a new treatment in combination with corticosteroids, immunosuppressive agents, or antiviral drugs during the follow-up were also classified as withdrawals and removed from the study. HCC was diagnosed by the presence of typical hypervascular characteristics on angiography, in addition to the findings on computed tomography and ultrasonography. Microscopic examination of fine-needle biopsy material was carried out in patients whose angiograms did not demonstrate a typical image of HCC.

Liver Histology

Liver biopsy specimens were obtained percutaneously or by peritoneoscopy using a modified Vim Silverman