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-

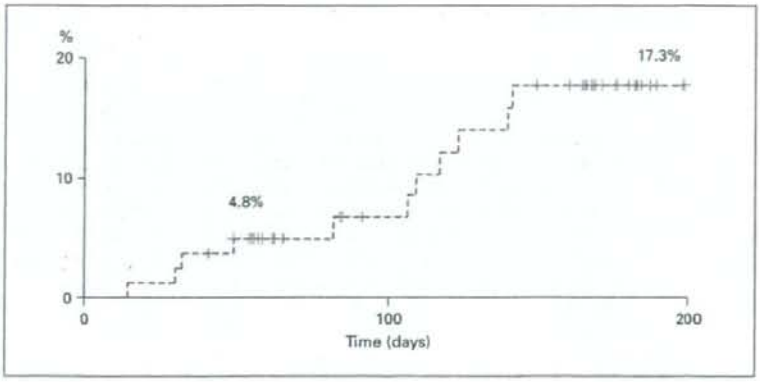

Fig. 1. Cumulative dropout rate due to adverse events during IFN therapy.

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 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

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.

Acknowledgments

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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

KENJI IKEDA, YASUJI ARASE, MASAHIRO KOBAYASHI, SATOSHI SAITOH, TAKASHI SOMEYA, TETSUYA HOSAKA, HITOMI SEZAKI, NORIO AKUTA, YOSHIYUKI SUZUKI, FUMITAKA SUZUKI, and HIROMITSU KUMADA

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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From the Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan, and Okinaka Memorial Institute for Medical Research, Tokyo, Japan.

Address for reprint requests: Kenji Ikeda, MD, Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo, 105-8470, Japan; ikedakenji@tora.email.ne.jp

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

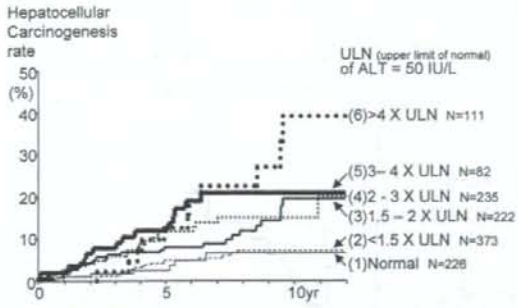

Fig 1. Carcinogenesis rates according to initial ALT values classified into six groups: (1) normal ALT, (2) <1.5 times ULN, (3) 1.5–2 times ULN, (4) 2–3 times ULN, (5) 3–4 times ULN, and (6) >4 times of ULN. The higher the average ALT, the higher the carcinogenesis rate was.

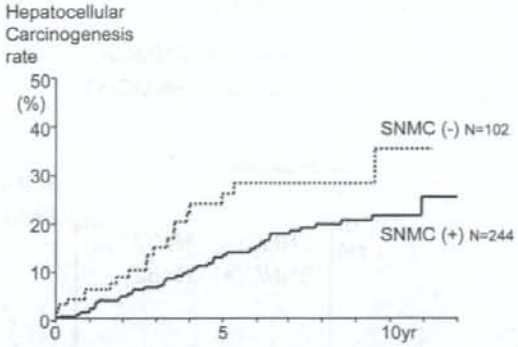

Fig 2. Carcinogenesis rates in patients with high average ALT values of twice or more of ULN, excluding those patients with biochemical responders who continued a normal ALT value at least 6 months just after IFN therapy. The carcinogenesis rate of glycyrrhizin-treated group was significantly lower than 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

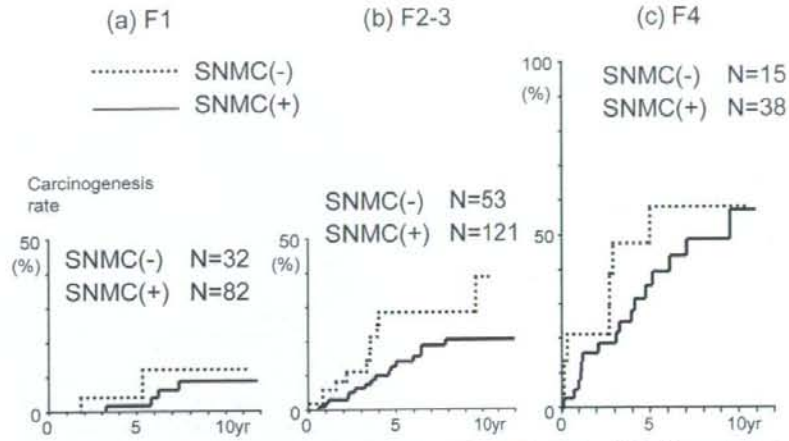

Fig 3. Carcinogenesis rates according to hepatitis staging: (a) F1 stage hepatitis, (b) F2–F3 stage hepatitis, and (c) F4 or cirrhotic stage. In each fibrotic stage of hepatitis, carcinogenesis rates were lower in the glycyrrhizin group than in the untreated group.

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.

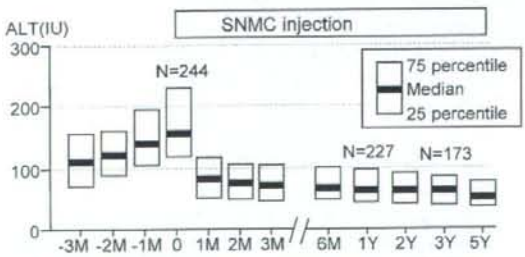

Fig 4. Aminotransferase activity before and after glycyrrhizin therapy. ALT value significantly decreased after the initiation of glycyrrhizin injection therapy.

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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Long-Term Follow-Up of HBeAg-Positive Young Adult Japanese Patients Treated with Corticosteroid Withdrawal Therapy for Chronic Hepatitis B

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

Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan

Key Words
Chronic hepatitis B · Corticosteroid withdrawal therapy · HBeAg seronegative rate

Abstract

Objectives: To evaluate the long-term effects of corticosteroid withdrawal therapy (CSWT) in young adult Japanese patients with chronic hepatitis B (CH-B) virus infection. ***Methods:*** The subjects were 106 patients with CH-B who received CSWT, were less than 35 years of age and had been followed for more 10 years after CSWT. ***Results:*** Retreatment was not required in 41 patients (38.7%; retreatment(–) group) while 65 (61.3%) received treatment after the initial CSWT (retreatment(+) group). Larger proportions of patients of the retreatment(–) group were females, had liver histology stage F2/F3, high ICG R15, and genotypes A/B/D/E, compared with the retreatment(+) group. At the last follow-up examination, the HBeAg seronegative rate was 90.2% in the retreatment(–) group and 98.5% in retreatment(+) group. In the retreatment(–) group, the rate of liver cirrhosis (LC; 7.3%, 3 patients) was lower, but the rate of hepatocellular carcinoma (HCC; 12.2%, 5 patients) was higher than in the retreatment(+) group (20%, 13 patients, and 4.6%, 3 patients, respectively). At the 10-year period, the overall HBsAg loss, LC and HCC rates were 2.8, 13.2 and 1.9%, respectively. ***Conclusions:*** Our results suggest that CSWT is good short-term therapy and has possible long-term effects in young adult Japanese patients with CH-B.

Introduction

Chronic hepatitis B (CH-B) is associated with high morbidity and mortality. It is estimated that 2 billion people worldwide have been infected with the hepatitis B virus (HBV), among whom more than 350 million have CH-B. Approximately 25–40% of them will develop hepatocellular carcinoma (HCC) and liver cirrhosis (LC) [1]. With regard to treatment for CH-B, we have used corticosteroid withdrawal therapy (CSWT) and interferon (IFN)-α, and recently nucleoside analogs such as lamivudine. The aims of any treatment are to inactivate liver disease as indicated by hepatitis B e antigen (HBeAg) seroconversion and disappearance of serum HBV DNA and to impede the progression of the pathological process and the development of LC/HCC.

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Hitomi Sezaki, NMD
Department of Gastroenterology, Toranomon Hospital
2-2-2 Toranomon, Minato-ku
Tokyo 105-8470 (Japan)
Tel. +81 44 877 5111, Fax +81 44 877 5333, E-Mail hitomis@mx1.harmonix.ne.jp

Previous studies reported the disappearance of HBV DNA, loss of HBeAg with or without seroconversion to anti-HBe, normalization of serum transaminase levels during the natural course of the disease [1, 2], CSWT [3–8] and IFN therapy [9–12] in patients with CH-B infection. For IFN therapy, the results of long-term follow-up studies have already been reported [10–12]. However, there is little or no information on the long-term effects of CSWT on disease progression and mortality in patients with CH-B infection.

To evaluate the long-term effects of CSWT on disease progression and mortality in chronic HBV-infected patients, we performed a retrospective study on HBeAg-positive CH-B patients, especially young adults who were less than 35 years old at the start of therapy, who received CSWT and were followed up for more than 10 years in our hospital.

Patients and Methods

From 1971 to 2002, a total of 193 CH-B patients who were less than 35 years of age received CSWT for the first time at Toranomon Hospital, Tokyo, Japan. The diagnosis of CH-B was based on the presence of hepatitis B surface antigen (HBsAg) for more than 6 months, liver biopsy and HBeAg positivity. The median follow-up period was 11.6 (range 0.2–32.9) years. To evaluate the long-term effects of CSWT in these patients, we selected only patients with a more than 10-year follow-up from the commencement of CSWT. Accordingly, 106 patients were enrolled in this study. They included 84 males and 22 females, aged 12–34 years, with a median age of 29 years. All patients were negative for anti-HCV antibody.

Treatment Protocol

Patients were treated with oral corticosteroid in a single dose of 40 mg/day for the 1st week, 30 mg/day for the 2nd week, 20 mg/day for the 3rd week, and then 10 mg/day for the last week. Then, 25 (23.6%) of them received IFN therapy within 4 weeks when a clinical rebound following CSWT and a tendency to increasing alanine aminotransferase (ALT) levels were observed within 3–5 weeks after discontinuation of CSWT. Clinical rebound after CSWT represented an increase above 5-fold the upper limit of normal ALT levels.

Patients were divided into 2 groups based on the need for retreatment: a group without retreatment (retreatment(–)) and a group with retreatment (retreatment(+)). Retreatment included CSWT, IFN therapy and nucleoside analog therapy. We regarded initiation of some kind of therapy more than 5 weeks after discontinuation of CSWT or administration of IFN for more than 4 weeks, even if within 4 weeks after discontinuation of CSWT, as retreatment.

Blood Tests

Routine biochemical and hematological tests were performed at each visit to our outpatient clinic during and after the first CSWT. The remaining serum samples were divided and stored at –80°C until the virological tests were performed. HBsAg was determined by hemagglutination, using commercially available kits (MyCell, Institute of Immunology, Tokyo, Japan), and HBeAg and antibody to HBeAg (anti-HBe) were measured using an enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (Abbot Diagnostics, Chicago, Ill., USA). HBV-DNA was assessed by a transcription-mediated amplification and hybridization protect assay (TMA; Chugai Diagnostics Science Co., Tokyo) [13] and Cobas Amplicor HBV Monitor Test (Amplicor; Roche Diagnostics, Branchburg, N.J., USA). The lower limit of the TMA assay was 3.7 LGE/ml and the lower limit of the Amplicor assay was 2.6 log copies/ml. Genotyping of HBV was performed by an ELISA kit (HBV Genotype ELISA, Institute of Immunology, Tokyo) using monoclonal antibodies for the genotype-specific epitopes in the pre-S2 region product [14].

Liver Histopathological Examination

Histopathological staging of the liver biopsy specimens was performed according to the classification of Desmet et al. [15].

Follow-Up

Data were collected by reviewing patient clinical records, survival, development of LC (defined as histopathological findings or the presence of ascites, encephalopathy and gastroesophageal varices), and HCC. Follow-up time was calculated from the start of CSWT until the last visit or death.

Statistical Analysis

Nonparametric tests, including the χ^2, Fisher exact probability and Mann-Whitney U tests, were used to analyze the background characteristics of patients. A p value of <0.05 was considered statistically significant. The Kaplan-Meier method was used to estimate the time to HBeAg seronegativity, HBsAg loss and development of LC and HCC. All analyses were performed using SPSS version 10.1 (SPSS Inc., Chicago, Ill., USA).

Results

Baseline Characteristics

The baseline characteristics of the patients at commencement of CSWT are shown in table 1. Of 106 patients, 41 (38.7%) did not receive any retreatment (retreatment(–) group) while the remaining 65 (61.3%) patients received some kind of retreatment (retreatment(+) group). There were no differences between the 2 groups with respect to age, serum ALT, total bilirubin, platelet count and HBV DNA levels. The ICG R15 level in the retreatment(–) group was higher than in the other group (table 1). The proportion of females in the retreatment(–) group was significantly higher than in the other group (39 vs. 9%, respectively; $p = 0.000$). Furthermore, the proportion of the retreatment(–) group with a pretreatment liver histopathology grade of F2/F3 was significantly higher than those of the retreatment(+) group (34 vs. 31%, respective-

Table 1. Baseline characteristics at the start of first CSWT

	Total (n = 106)	Retreatment(–) (n = 41)	Retreatment(+) (n = 65)	p value
Follow-up, years[a]	15.6 (10.2–32.9)	14.7 (10.2–32.9)	16.3 (10.5–25.6)	
Age, years	29 (12–34)	28 (12–34)	29 (13–34)	
Sex, male/female	84/22	25/16	59/6	0.000
Family history of liver disease	76 (71.0%)	30 (73.1%)	46 (69.7%)	
Histology, F1/2/3	64/26/8	23/10/4	41/16/4	0.002[b]
ALT, IU/l	380 (48–835)	384 (64–746)	370 (48–835)	
T-Bil, mg/dl	0.7 (0.2–2.0)	0.7 (0.4–2.0)	0.7 (0.2–1.9)	
Platelets, $\times 10^3/\mu l$	19.6 (9.9–51.5)	18.8 (9.9–30.0)	20.3 (11.8–51.5)	
ICG R15, %	13 (2–29)	16 (4–29)	12 (2–27)	0.007
HBV DNA, LGE/ml	8.2 (<3.7–8.7<)	8.1 (<3.7–8.7<)	8.4 (6.6–8.7<)	
HBV genotype, A/B/C/D(E)/unknown	4/5/93/1/3	3/2/34/1/1	1/3/59/0/2	0.000[c]

ALT = Alanine aminotransferase; T-Bil = total bilirubin; ICG R15 = indocyanine green retention rate at 15 min.

[a] Data are presented as median (range).

[b] p value was compared F1 with except F1.

[c] p value was compared C with except C.

Table 2. Comparison of HBeAg seronegative rate in patients with or without retreatment

	Retreatment(–) (n = 41)	Retreatment(+) (n = 65)
HBeAg seronegative rate after first CSWT	38 (92.7%)	34 (52.3%)[a]
Period until HBeAg seronegative, years[b]	1.0 (0.0–15.5)	1.1 (0.1–10.4)
HBeAg positive re-conversion rate	19/38 (50.0%)	20/34 (58.8%)
HBeAg re-seronegative rate	18/19 (94.7%)	6/20 (30.0%)
HBeAg seronegative rate within a year after CSWT	19 (46.3%)	15 (23.1%)
HBeAg seronegative rate at last observation	37 (90.2%)	64 (98.5%)
Period until last HBeAg seronegative, years	5.4 (0.0–15.5)	5.8 (0.1–19.4)

[a] Retreatment cases were assessed before retreatment.

[b] Median value (range).

ly; p = 0.002). The proportion of the retreatment(–) group with genotype A, B, D, E or unknown (17%) was significantly higher than that of the retreatment(+) group (9%, p = 0.000). The median follow-up period for the whole group was 15.6 (range 10.2–32.9) years, and was not different between the 2 groups (table 1).

Comparison of HBeAg Seronegative Rates

We also examined HBeAg seronegative conversion rates in the 2 groups (table 2). HBeAg seronegative rate after the first CSWT was higher in the retreatment(–) than the retreatment(+) group. In particular, the proportion of the retreatment(–) group with an early HBeAg seronegative rate (within 1 year after CSWT) was higher than that of the retreatment(+) group (table 2). Although about half of both groups became positive again for HBeAg, 94.7% of the retreatment(–) group later spontaneously converted to seronegativity. In comparison, only 30.0% of the retreatment(+) group converted spontaneously to seronegativity (which explains why they needed retreatment). However, the HBeAg seronegative rates were high in both groups at the last observation (table 2).

Table 3. Comparison of prognosis in patients with or without retreatment after first CSWT

	Retreatment(–) (n = 41)	Retreatment(+), final treatment (n = 65)			Total (n = 106)
		CSWT (n = 9)	IFN (n = 34)	lamivudine (n = 22)	
ALT normalization	34 (82.9%)	7 (77.8%)	29 (85.3%)	19 (86.4%)	90 (84.1%)
HBeAg seronegative	37 (90.2%)	9 (100%)	33 (97.1%)	22 (100%)	101 (95.2%)
HBV DNA negative[a]	10 (24.4%)	1 (12.5%)	14 (46.7%)	12 (54.5%)	37 (34.9%)
HBsAg loss	4 (9.8%)	2 (22.2%)	5 (14.7%)	0	11 (10.4%)
Development of LC	3 (7.3%)	1 (11.1%)	7 (20.6%)	5 (22.7%)	16 (15.1%)
Development of HCC	5 (12.2%)	0	3 (8.8%)	0	8 (7.5%)
Death	5 (12.2%)	0	0	0	5 (4.7%)

[a] In cases measured by Amplicor assay, <2.6 log copies/ml was considered as less than sensibility.

Prognosis after First CSWT

Table 3 summarizes the effect of retreatment or no retreatment at the last observation. For the whole group, the ALT normalization rate and HBeAg seronegative rate were high. In particular, the HBeAg seronegative rate and HBV DNA negative (less than sensitivity, <2.6 log copies/ml) rate in the lamivudine therapy group were the highest among the groups. On the other hand, the HBsAg loss rate was highest in patients with final retreatment by CSWT, followed by patients with final retreatment by IFN, retreatment(–) group, while none of the patients with final retreatment by lamivudine showed HBsAg loss. Furthermore, the proportion of patients in the retreatment(–) group who developed LC (7.3%) was lower than that of patients in the retreatment(+) group (13 of 65 patients, 20%). On the other hand, the proportion of patients in the retreatment(–) group who developed HCC (12.2%) was higher than that of the retreatment(+) group (3 of 65 patients, 4.6%). Only 5 deaths were recorded during the follow-up and all of them were in the retreatment(–) group (table 3). Death was due to HCC in 4 cases and other illness in the remaining patient.

Time to HBeAg Seronegativity, HBsAg Loss, Development of LC and HCC

Figure 1 shows the time to HBeAg seronegativity, HBsAg loss, development of LC and HCC in all patients aged less than 35 years and who received CSWT. The HBeAg seronegative rate increased progressively from 1 to 15 years. However, the increase in the rate of HBsAg loss was less each year up to 20 years of follow-up. The rates of development of LC and HCC during the 20-year follow-up period were also low (fig. 1).

Discussion

The main goals of treatment for patients with CH-B are loss of HBeAg and normalization of aminotransferase. Various treatments such as CSWT, IFN, nucleoside analogs have been used, and their effects have been discussed [3–12]. Previous studies reported the short-term effects of CSWT [3–5, 7]. We also reported previously the short-term effects of CSWT; the HBeAg seronegative rate was 70% within 1 year after CSWT [3]. In that report, the age of the subjects was 39.0 ± 9.9 years. In the present study, we evaluated the effect of therapy in younger adult patients (<35 years) with CH-B. In addition, the study was designed to evaluate the long-term effects and prognosis of CSWT, and accordingly only patients who were followed up for more than 10 years after CSWT were selected.

In the present study, the proportion of patients who did not need retreatment after the first CSWT was 38.7%, and 38 of them (92.7%) became HBeAg seronegative. The HBeAg seronegative rate in patients who needed retreatment after the first CSWT was obviously lower (34/65, 52.3%). However, in both groups the median period until HBeAg seronegativity was approximately 1.0 year; and thus, the HBeAg seronegative rate within 1 year after CSWT was almost the same in the 2 groups; 46.3 (19/38) and 44.1% (15/34) in the HBeAg seronegative cases of the retreatment(–) and retreatment(+) groups, respectively. Of 72 cases who became HBeAg seronegative once, 39 cases (54%) converted to HBeAg positivity, and this rate was almost similar in both groups. During the later part of the follow-up period, though HBeAg changed spontaneously to become negative again at a high rate (94.7%) in the retreatment(–) group, the HBeAg re-seronegative

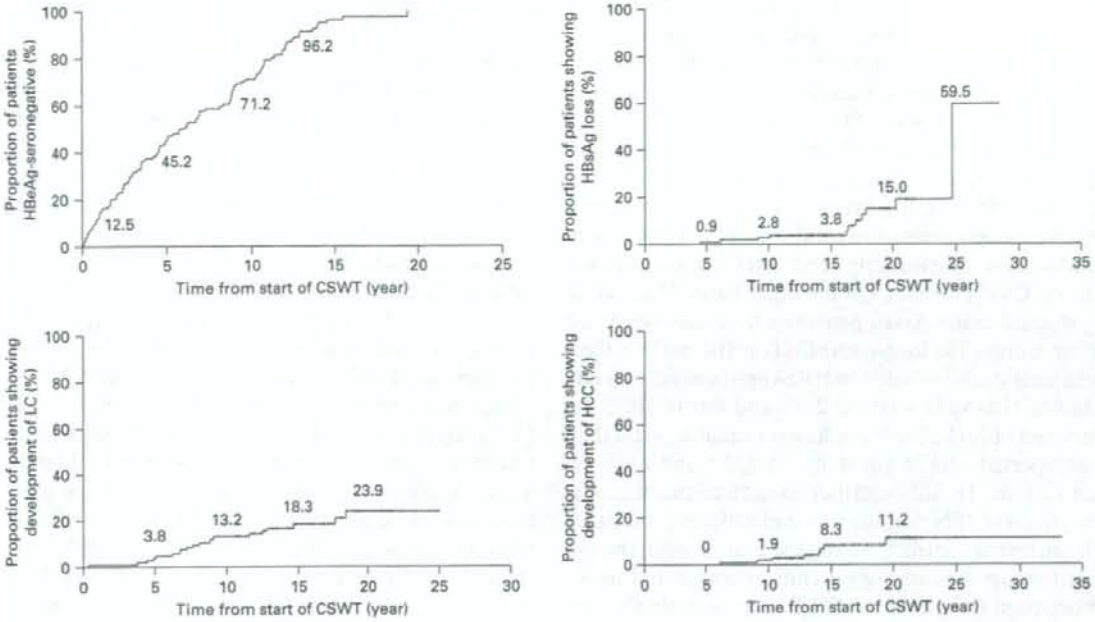

Fig. 1. Time to HBeAg seronegativity, HBsAg loss, development of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) after corticosteroid withdrawal therapy (CSWT) calculated for all patients who participated in the study.

rate was low in the retreatment(+) group (30.0%). Considering the difference in the clinical course, it is suggested to be different according to whether patients achieve HBeAg seronegative easily. The retreatment(–) group contained more females and more patients of genotypes A/B/D/E/unknown than the other group. One reason for the high female ratio in the retreatment(–) group is possibly pregnancy, continuation of therapy was difficult in these patients. In addition, as reported previously [16], the clinical course in female patients with CH-B is often better than in their male counterparts. On the other hand, in terms of HBV genotypes, Kao et al. [17, 18] reported that HBV genotype C is associated with a lower response rate to IFN-α therapy compared with genotype B. In the present study, although there were only a few patients with genotype B, we speculate that genotype C, compared with others except for genotypes C, may be associated with greater resistance to CSWT. However, the HBeAg seronegative rate was 90.2% in the retreatment(–) group at the last observation. On the other hand, in the retreatment(+) group, although their spontaneous HBeAg seroconversion rate was also low, repeating administration of CSWT, IFN, or lamivudine in fact markedly increased the rate to 98.5%, which was even higher than that of the retreatment(–) group (table 2). In addition, as shown in table 3, the ALT normalization rate and HBV DNA negative rate in the retreatment(+) group were also equal or higher than those of the retreatment(–) group. Considered together, the present results suggest that even if the response to the first CSWT is not satisfactory, good virological effects can be provided by repeating or providing alternative treatments.

Although there are no studies that evaluated the long-term effects of CSWT in CH-B, several studies evaluated the long-term effects of IFN therapy. Niederau et al. [10], Yuen et al. [11] and van Zonneveld et al. [12] reported the long-term effect of IFN therapy, although the follow-up period was shorter than ours, including evaluation of HBeAg seroconversion rate, HBsAg loss rate and development rate of LC and HCC. Therefore, we compared the results of these studies with ours. In the study by Niederau et al. [10], the HBeAg seroconversion rate and

HBsAg loss rate were higher than those of our CSWT patients (56 vs. 45.2% at 5 years and 11.6 vs. 0.9%, respectively). The reasons for the differences are possibly race-related (they evaluated Caucasian patients) and the genotypes of their cases were different from ours. The study by van Zonneveld et al. [12] also included predominantly Caucasian patients and reported that the HBsAg loss rate was 21.8% and the rate of HCC development was 4.8% (we grouped responders and non-responders together), which are considerably good results in comparison with our CSWT results. On the other hand, Yuen et al. [11] studied many Asian patients whose ages were also similar to ours. The long-term effects at 10-year IFN therapy in their study included an HBeAg seroconversion rate of 43.8%, HBsAg loss rate of 2.4% and rate of HCC development of 2.4%. Their results were equal or better than those reported here in our study, 71.2, 2.8 and 1.9%, respectively (fig. 1). Although they considered that the long-term effects of IFN therapy were not different compared with untreated controls, we suggest that because the untreated group showed a good clinical course and hence did not need treatment for CH-B, it is possible that the clinical course of both groups was not different. We compared the long-term effects in the retreatment(–) group and retreatment(+) group after first CSWT in our patients. The ALT normalization rate, HBeAg seronegative rate, HBV DNA negative rate and HBsAg loss rate were not different between the 2 groups. These results suggest that because the retreatment(–) group did not need retreatment based on the good clinical course and the retreatment(+) group required repeated treatments for CH-B, the virological effects could become almost equal at the final observation.

The rates of development of LC and HCC were not similar in the 2 groups. With regard to the development of LC, although the progression of fibrosis after the first CSWT was more severe in the retreatment(–) group, the rate of development of LC was lower than in the retreatment(+) group. The reason for this finding is probably related to the good clinical course after CSWT as they did not need retreatment. On the other hand, the rate of development of HCC in the retreatment(–) group was higher than in the retreatment(+) group. Although the incidence of complications was low most likely due to the young age of our patients, we found disaggregation between the LC development rate and carcinogenic rate. Ikeda et al. [19] used multivariate analysis and showed that the severity of fibrosis in HBV-related chronic hepatitis was not associated with the development of HCC. Another clinicopathologic study of HCCs in chronic HBV carriers revealed that about 20–50% of such patients do not have accompanying cirrhosis [20]. As we previously stated in patients with HBV infection at a young age who later developed HCC without LC [21] and previous studies that showed HBV DNA integration into the cellular genomic DNA in HCC cases [22–25], we speculate that the development of HCC in our cases without LC might also be associated with HBV DNA integration into the cellular genomic DNA.

The choice of therapy for young patients with CH-B is difficult. The aim of therapy is HBeAg to anti-HBe seroconversion and inactivation of the disease process. However, despite administration of the same therapy, some patients show good response to one course of CSWT, while others repeatedly require other treatments for CH-B. However, because of the age of young patients with HBV infection in patients like ours, it is important to provide a good quality of life, including cessation of all medications at some stage of their lives. Comparison of our results with those of other studies that used IFN therapy showed that the effects of CSWT in long-term follow-up was almost equal to that of IFN. Although nucleoside analogs such as lamivudine are good antiviral agents, their long-term effects are still unclear especially when treatment can be finished in the short-term and the problem associated with long-term induced lamivudine-resistant mutation and breakthrough hepatitis. Therefore, it is important to examine the long-term effects and safety of IFN therapy and nucleoside analogs such as lamivudine.

In conclusion, we evaluated the long-term effects of CSWT in young adult Japanese HBeAg-positive patients. In patients less than 35 years of age who received CSWT for the first time, 38.7% did not need retreatment with good virological effects. The main results are: (1) the retreatment(–) group consisted of more females and more patients infected with hepatitis B virus than another genotype except for C who showed good response to CSWT and HBeAg might easily become seronegative; (2) the overall long-term effects of CSWT on HBeAg seroconversion rate, HBsAg loss rate and rate of HCC development were equal or better compared with previous reports of IFN therapy [11], and (3) the rate of HCC development was high in the retreatment(+) group compared with the rate of LC development. We speculate that the high rate is probably due to HBV DNA integration into the cellular genomic DNA. Our results suggest that CSWT is a good short-term therapy with possible long-term effects for young adult Japanese patients with CH-B. Other studies should also evaluate the long-term effects of IFN and nucleoside analogs such as lamivudine therapy in Japan.

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Emergence of a Novel Lamivudine-Resistant Hepatitis B Virus Variant with a Substitution Outside the YMDD Motif[▽]

Hiromi Yatsuji,[1,2] Chiemi Noguchi,[1,2] Nobuhiko Hiraga,[1,2] Nami Mori,[1,2] Masataka Tsuge,[1,2] Michio Imamura,[1,2] Shoichi Takahashi,[1,2] Eiji Iwao,[3] Yoshifumi Fujimoto,[2,4] Hidenori Ochi,[2,4] Hiromi Abe,[1,4] Toshiro Maekawa,[4] Chise Tateno,[2,5] Katsutoshi Yoshizato,[2,5,6] Fumitaka Suzuki,[7] Hiromitsu Kumada,[7] and Kazuaki Chayama[1,2,4]*

Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima-shi, 734-8551, Japan[1]; Liver Research Project Center, Hiroshima University, Hiroshima, Japan[2]; Pharmaceuticals Research Unit, Mitsubishi Pharma Corporation, 1000 Kamoshida-cho, Aoba-ku, Yokohama 227-0033, Japan[3]; Laboratory for Liver Disease, SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Yokohama 230-0045, Japan[4]; Yoshizato Project, CLUSTER, and Hiroshima Prefectural Institute of Industrial Science and Technology, Higashihiroshima, Japan[5]; Developmental Biology Laboratory, Department of Biological Science, Graduate School of Science, Hiroshima University, Higashihiroshima, Japan[6]; and Department of Gastroenterology, Toranomon Hospital, Tokyo, Japan[7]

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Lamivudine is a major drug approved for treatment of chronic hepatitis B virus (HBV) infection. Emergence of drug-resistant mutants with amino acid substitutions in the YMDD motif is a well-documented problem during long-term lamivudine therapy. Here we report a novel lamivudine-resistant strain of HBV with an intact YMDD motif, which included an amino acid substitution, rtA181T, in the reverse transcriptase (RT) domain of HBV polymerase. The substitution also induced a unique amino acid substitution (W172L) in the overlapping hepatitis B surface (HBs) protein. The YMDD mutant strains were not detected even by using the sensitive peptide nucleic acid-mediated PCR clamping method. The detected nucleotide substitution was accompanied by the emergence of an additional nucleotide substitution that induced amino acid change (S331C) in the spacer domain. The rtA181T mutant strain displayed a threefold decrease in susceptibility to lamivudine in in vitro experiments in comparison with the wild type. In vivo analysis using human hepatocyte-chimeric mice confirmed the resistance of this mutant strain to lamivudine. We developed a method to detect this novel rtA181T mutation and a previously reported rtA181T mutation with the HBs stop codon using restriction fragment length polymorphism PCR and identified one patient with the latter pattern among 40 patients with lamivudine resistance. In conclusion, although the incidence is not high, we have to be careful regarding the emergence of lamivudine-resistant mutant strains with intact YMDD motif.

Hepatitis B virus (HBV) is a small, enveloped DNA virus that causes chronic hepatitis and often leads to cirrhosis and hepatocellular carcinoma (4, 12, 33). To date, interferon and three nucleoside and nucleotide analogs (lamivudine, adefovir dipivoxil, and entecavir) have been approved by the United States Food and Drug Administration for the treatment of chronic HBV infection. Lamivudine, an oral cytosine nucleoside analogue, potently inhibits HBV replication by interfering with RNA-dependent DNA polymerase (10, 16, 22). Lamivudine therapy suppresses HBV replication in most patients and improves transaminase levels and liver histology (16, 22, 25, 30). However, prolonged therapy results in the emergence of drug-resistant mutants in 24% and 70% of patients after 1 and 4 years of therapy, respectively, followed by increases in viral load and re-elevation of transaminase levels (18).

* Corresponding author. Mailing address: Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Phone: 81-82-257-5190. Fax: 81-82-255-6220. E-mail: chayama@hiroshima-u.ac.jp.

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Most lamivudine-resistant strains show amino acid substitutions in the YMDD (tyrosine-methionine-aspartate-aspartate) motif in the C domain of HBV polymerase. In addition to the emergence of the YMDD mutation, rtL180M and rtV173L mutations in the B domain of HBV polymerase are frequently observed (1, 9). In vitro analyses have confirmed that the rtL180M mutation augments the level of lamivudine resistance and enhances viral replication, while the rtV173L mutation enhances only viral replication (9, 23). On the other hand, only a few uncommon mutations associated with lamivudine resistance have been reported so far (3, 7, 24, 34). In the C domain of HBV polymerase, rtM204S and rtD205N were detected in patients with lamivudine resistance (3, 7). In the B domain, rtL180C and rtA181T were associated with lamivudine resistance (7, 24, 34). Yeh et al. (34) reported the emergence of rtA181T mutants in 4 of 23 patients who received long-term lamivudine therapy. The mutant appeared concomitantly with or after emergence of YMDD motif mutants and persisted thereafter. The nucleotide substitution in the FLLA motif resulted in early termination of the overlapping HBs gene transcription by creating a stop codon (TGG to TGA). Yeh et al. (34) demonstrated that the mutation reduced the