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Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients

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Background/Aim: **Hepatocellular carcinoma frequently develops during the advanced stages of chronic hepatitis C. We examined whether interferon prevents the development of hepatocellular carcinoma in chronic hepatitis C patients.**

Methods: **Japanese patients with chronic hepatitis C (n=1.148; 117 with portal fibrous expansion (F1), 636 with bridging fibrosis (F2), 355 with bridging fibrosis and architectural distortion (F3)) and 40 cirrhotic (F4) patients were treated with interferon. These patients were followed from 1 to 7 years after interferon therapy. Blood tests and image analysis were serially performed to assess response to interferon and to detect hepatocellular carcinoma. Fifty-five cirrhotic type C patients (control F4) not receiving interferon were enrolled in this study.**

Results: **Sustained (SR: 27.5%) and transient (TR: 23.0%) responders totaled 50.5%, while 49.5% did not respond to interferon. SR showed an improvement in disease stage reflected by increased platelet counts. Fifty-two patients (9 F2, 36 F3, and 7 F4) developed hepatocellular carcinoma in the follow-up period; 3 SR, 8 TR, and 41 non-responders (NR). The cumulative incidence of hepatocellular carcinoma in F2 was significantly lower (p=0.019) in SR compared with NR, but not in SR in F3 and F4 patients. However, the cumulative incidence of hepatocellular carcinoma was significantly decreased in all SR (p=0.0001) and TR (p=0.0397) compared with all NR.**

Conclusion: **These results indicate that interferon therapy in chronic hepatitis C patients lowered the rate of progression of hepatocellular carcinoma in sensitive cases but not in patients in an advanced stage.**

Key words: **Hepatitis C virus infection; Hepatocellular carcinoma; Interferon therapy; Non-responder; Sustained responder; Transient responder.**

In 1996, AROUND 30 000 patients died of hepatocellular carcinoma (HCC) in Japan, where this tumor is the third leading cause of cancer deaths. Of these patients, 80–85% of patients were positive for anti-hepatitis C virus (HCV) antibody (1,2). Eighty to ninety percent of these patients had cirrhosis (1–4). The remaining patients had advanced stages of chronic hepatitis. HCC develops in these patients after years of hepatic inflammation. Thus, it is reasonable to consider that inhibiting the progression of chronic hepatitis may decrease the development of HCC.

Interferon (INF) alpha and beta have been widely used for treatment of chronic hepatitis type C (CH-C) since public health insurance began to cover this therapy in January 1992. Thirty to forty percent of patients showed a sustained response after IFN (5,6). To assess if IFN reduces the development of HCC, we compared the annual incidence and cumulative rate of HCC in sustained responders, transient responders, and non-responders among CH-C patients who received IFN.

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Materials and Methods

Patients and treatment

Patients who were positive for hepatitis B surface antigen or had a history of heavy drinking (daily alcohol intake more than 60 g of ethanol for more than 5 years) were excluded from this study. One thousand two hundred and ninety-nine Japanese CH-C patients received IFN therapy between January 1989 and September 1995 at the 13 hospitals of the Viral Hepatitis Study Groups listed in the Appendix to this paper, the University Hospital, and Kyoto Prefectural Yosanoumi Hospital, of whom 1148 patients were followed up for more than 1 year after IFN treatment. The other 151 patients did not visit the 15 hospitals after IFN therapy, even when letters were sent to them requiring them to attend for consultation at one of the hospitals. Patients less than 18 years old and over 68 years old were excluded from this study.

All patients were positive for serum anti-HCV antibody by a first- or second-generation enzyme-linked immunosorbent assay (ELISA; Ortho Diagnostics, Tokyo, Japan) and for serum HCV RNA. Serum HCV RNA was detected using reverse transcriptase-polymerase chain reaction (RT-nested PCR) as described previously (7). Patients were negative for antibodies against human immunodeficiency virus.

Among the cirrhotic type C patients visiting the Kyoto Prefectural University Hospital from 1987 to 1995, 55 cirrhotic patients who had not received IFN were enrolled as controls (control F4). The criteria for the control cirrhotic patients were as follows: less than 68 years old, not heavy drinkers, less than 2 mg/dl in total serum bilirubin, no ascites, no HCC by ultrasonography (US) and computed tomography (CT) at entry, and followed up for more than 2 years.

Serum alanine aminotransferase (ALT), blood cell counts, and serum alpha fetoprotein (AFP) were measured before IFN therapy, and in two-thirds of the patients the amount of serum HCV RNA and HCV genotype were determined before IFN therapy. US was performed in all patients before therapy to document the absence of space-occupying lesions suggesting HCC. Serum ALT, platelet (PLT) counts, and AFP were serially checked during the follow-up period.

All patients underwent liver biopsy prior to IFN treatment and all cirrhotic patients underwent laparoscopy and/or liver biopsy. Histologic criteria and staging of chronic hepatitis were based on the new classification by Desmet et al. (8,9) in which staging is defined as follows: F0 (no fibrosis), F1 (fibrous portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion), and F4 (cirrhosis). One hundred and seventeen patients (male: 70, female: 47, age: 42.6±13.2 years) were in the F1 stage prior to IFN therapy, 636 (male: 413, female: 223, age: 49.5±11.2 years) were in F2, 355 (male: 223, female: 132, age: 54.7±9.1 years) were in F3, and 40 (male: 18, female: 22, age: 55.6±8.3 years) were in F4. In the control F4, average age was 57.6±5.2 years: 28 were male and 27 were female, with average ages 58.6±4.5 years and 57.7±5.7 years, respectively. Of the males, 11 were <60 years and 17 were ≧60 years. Of the females, 19 were <60 years, and 11 were ≧60 years. Serum ALT levels and PLT counts in each stage are shown in Table 1. Serum ALT levels increased, and PLT counts decreased in proportion to the progress of the stage of liver disease (Table 1).

Of the 1148 patients, 582 received natural IFN alpha (nIFNα), 204 received recombinant IFN alpha 2a (rIFNα 2a), and 362 received recombinant IFN alpha 2b (rIFNα 2b). Patients received 3 MU to 10 MU of IFNα intramuscularly daily or thrice a week for 16 to 24 weeks; the total amount of IFN was 252 to 780 mega units (MU). In principle, the schedule of IFN therapy was daily injection of IFN for the initial 2 weeks and then three times a week; however, administration of interferon was changed to three times a week if marked leukopenia (<2000/mm^3) or thrombocytopenia (<30 000 mm^3) was seen during IFN therapy. All F4 patients received 3 or 6 MU of IFNα daily for the initial 2 weeks and then three times a week for 22 to 24 weeks.

Written informed consent to participate in these trials was obtained from all patients, and all aspects of these studies were approved by the Ethical Committees of the 15 participating hospitals.

The patients were classified into three groups according to the response to IFN therapy. Sustained responders (SR) were defined as patients with normal serum ALT levels (≦30 IU/l) for more than 6 months after IFN treatment. Transient responders (TR) were defined as patients with normal ALT at the end of treatment which then increased to >30 IU/l after IFN treatment. Non-responders (NR) had elevated serum ALT levels despite IFN therapy. Patient demographics are shown in Table 1.

Follow-up study

The follow-up schedule was different for each stage. All patients had serum chemistry determinations every month during the initial 6 months, and serum AFP was checked every 3 months in F2, F3, and F4 patients. After IFN therapy, TR and NR patients had blood chemistries performed every 1 to 2 months, while SR patients were checked every 1 to 3 months. Serum HCV RNA was examined at the end of treatment and then 6 and 24 months after IFN therapy. Of the 1148 patients, 514 were mainly followed by their practitioners but 271 of the 514 patients had no consultation at any of the 15 hospitals for more than 1 year during the follow-up period. We sent those patients letters on July 1996 requiring them to attend for consultation at one of the 15 hospitals and to undergo image studying.

In principle, US was performed once a year for F1 patients, twice yearly for F2, and three times a year for F3, F4, and control F4 patients. The 271 patients did not come in for imaging studies during the follow-up period for early detection of HCC. In principle, CT was done once a year for F2 and twice yearly for F3, F4, and control F4. Dynamic CT and/or magnetic resonance imaging (MRI) were done when hepatic space-occupying lesions were found by US. To obtain a diagnosis of HCC, angiography and/or US-guided tumor biopsy were

TABLE 1

Characteristics of patients with chronic liver diseases type C at baseline

Stage of liver disease (sex)	Age[1] (years)	ALT[2] (IU/l)	PLT count[3] (×104/ml)	Follow-up period[4] (months)
F1 (n=117) (70 M, 47 F)	42.6±13.2	92.3±78.5	21.0±5.2	42.0±17.8
F2 (n=636) (413 M, 223 F)	49.5±11.2	114.8±97.5	18.0±5.1	39.5±13.9
F3 (n=355) (223 M, 132 F)	54.7±9.1	129.2±92.2	14.6±4.2	41.0±13.2
F4 (n=40) (18 M, 22 F)	55.6±8.3	107.1±41.8	9.7±2.5	45.7±12.0
Control F4 (n=55) (28 M, 27 F)	57.6±5.2	118.2±38.4	11.1±2.0	67.1±28.3

Data are expressed as mean±SD.

Tukey's q-test was used for the statistical analysis of age, ALT, PLT count, and follow-up period.

[1] (F1 *vs* F2, F2 *vs* F3; p=0.00001, F3 *vs* F4, p=0.95160).

[2] (F1 *vs* F2; p=0.07617, F2 *vs* F3; p=0.09126, F3 *vs* F4; p=0.48092).

[3] (F1 *vs* F2; p=0.00030, F2 *vs* F3; p=0.00043, F3 *vs* F4; p=0.90215).

[4] No significant differences were noted in their follow-up periods among the 4 groups.

TABLE 2

Effects of interferon therapy on chronic liver disease type C

Stage of liver disease	Response of interferon therapy		
	SR	TR	NR
F1 (n=117)	47 (40.2%)[1]	39 (33%)	31 (26.5%)
F2 (n=636)	194 (30.5%)[2]	156 (24.5%)	286 (45.0%)
F3 (n=355)	73 (20.6%)[3]	62 (17.5%)	220 (62.0%)
F4 (n=40)	2 (5.0%)	7 (17.5%)	31 (77.5%)

SR: sustained responder, TR: transient responder, NR: non-responder. [1] p=0.0008 (F1 *vs* F2), [2] p=0.001 (F2 *vs* F3), and [3] p=0.0269 (F3 *vs* F4) when Wilcoxon's rank-sum test was used for statistical analysis.

performed in all patients with suspected HCC by image analysis or when significant elevations of serum AFP occurred.

Mean follow-up periods were 42.0±17.8 months in F1, 39.5±13.9 months in F2, 41.0±13.2 months in F3, 45.7±12.0 months in F4, and 67.1±28.3 months in control F4 (59.2±30.9 months in males, 70.7±24.6 months in females) (Table 1). No significant differences in the duration of follow-up periods were noted among the F1, F2, F3, and F4 groups; however, the follow-up period of control F4 was significantly (p=0.0000046) longer than F4.

Statistical methods

SAS software (SAS Institute Inc.) was used for statistical analysis. Tukey's q-test was used to evaluate differences in age, serum ALT level, and PLT counts among the four groups (F1, F2, F3, and F4) prior to IFN therapy. Differences in the follow-up periods were compared by means of Student's t-test. Wilcoxon's rank-sum test was used to evaluate differences in the efficacy of IFN therapy and the sex ratios in the four groups. Wilcoxon's rank-sum test was used to estimate differences in the changes in PLT counts before and 2 years after IFN therapy in SR and NR. Cox proportional hazard model was used to evaluate whether sex, age, stage of liver disease, serum ALT level, and PLT count are risk factors of hepatocarcinogenesis. The cumulative incidences of HCC among total SR, TR, and NR in the four groups were evaluated using the log-rank test. The cumulative incidences of HCC among SR, TR, and NR in the four groups and the control F4 were also evaluated using the log-rank test.

Results

Response to IFN therapy

The distribution of response in each stage of liver disease is shown in Table 2. The rate of SR was well correlated with the stage of liver disease (F1 *vs* F2; p=0.0008, F2 *vs* F3; p=0.0001, F3 *vs* F4; p=0.0269). No significant differences were noted in the response to IFN therapy between males and females in any group.

Among the SR in each group of patients, two of the 40 SR in F1, 12 of the 156 SR in F2, and six of the 61 SR in F3 were positive for serum HCV RNA 6 months after the cessation of IFN therapy. Of these 20 SR with hepatitis C viremia, four patients became symptomatic during the follow-up period: 11, 14, 20, and 24 months after IFN therapy, respectively.

Platelet counts

PLT counts were significantly (p<0.01) lower with the progression of liver disease (F1 *vs* F2, F2 *vs* F3, and F3 *vs* F4). PLT counts were compared before and 24 months after IFN therapy in all groups (Table 3). Platelet counts were significantly (p=0.0001) increased in SR in F2 and F3 patients 2 years after IFN therapy. In NR, PLT counts were significantly decreased in F2 (p=0.0171), F3 (p=0.0053), and F4 (p=0.0372) 2 years after IFN therapy compared with their counts before IFN therapy.

Serum AFP levels before IFN therapy

Of the 1148 patients two cases showed levels of more than 100 ng/ml of serum AFP at the start of IFN therapy. However, these serum levels of AFP decreased less than 50 ng/ml during IFN treatment.

TABLE 3

Changes of platelet count after interferon (IFN) therapy in chronic liver disease type C

Stage of liver disease	Effect of IFN therapy	No. of cases	Platelet count (10^4/ml)	
			Before IFN therapy	2 years after IFN therapy
F1 (n=95)	SR	40	20.5±5.5	21.3±5.2
	TR	30	21.2±5.1	21.9±5.5
	NR	25	21.6±4.9	20.5±5.9
F2 (n=523)	SR	156	18.4±5.1	19.8±5.0[1]
	TR	125	18.6±4.7	18.2±4.7
	NR	242	17.4±5.2	16.9±5.0[2]
F3 (n=302)	SR	61	15.0±4.3	17.7±4.6[1]
	TR	54	14.6±4.5	14.1±4.8
	NR	187	14.4±4.1	13.9±4.5[3]
F4 (n=35)	SR	2	9.9±3.3	13.2±7.4
	TR	6	10.6±3.7	10.3±3.1
	NR	27	9.7±2.5	8.3±2.2[4]

Wilcoxon's signed-rank test was used to evaluate the difference in PLT counts before and 2 years after IFN therapy ([1] p=0.0001, [2] p=0.017, [3] p=0.0053, [4] p=0.0372).

TABLE 4

Hepatocellular carcinoma in chronic hepatitis and liver cirrhosis type C after interferon therapy and in control cirrhosis type C

	Hepatocellular carcinoma in each stage				
	F2 (n=9)	F3 (n=36)	F4 (n=7)	Total (n=52)	Control F4 (n=20)
Male/female	8/1	11/25	4/3	37/15	13/7
Age (years old)[1]	47–67 (59.0±8.1)	51–70 (61.2±5.6)	54–68 (62.4±4.5)	47–70 (61.2±6.0)	55–74 (65.6±4.9)
No. of HCC in SR/TR/NR	0/1/8	3/5/8	0/1/6	3/7/42	
Follow-up period[1] (months)	13–52 (32.3±13.4)	14–61 (32.3±2.9)	14–48 (32.1±13.0)	13–61 (32.4±12.9)	26–120 (67.1±28.3)
Serum AFP level[1] (ng/ml)	1–137 (27.5±41.9)	2–824 (95.8±196.5)	13–66 (28.6±19.6)	1–824 (75.4±166.8)	12–398 (122.3±139.4)
Tumor size[1] (mm)	9–30 (20.9±7.6)	8–45 (18.6±9.2)	8–43 (19.1±12.7)	8–45 (19.4±9.6)	12–37 (25.1±10.8)

[1] At the time of detection of hepatocellular carcinoma (HCC).

Incidence of hepatocellular carcinoma

HCC was detected in 52 patients after IFN treatment (Table 4); 15 were detected within 24 months, and 37 were detected 24 to 61 months after IFN therapy. Of the 52 cases, one (0.6%) was detected in the 156 TR of F2, eight (2.8%) of 286 NR of F2, three (4.1%) of 73 SR of F3, five (8.1%) of 62 TR of F3, and 28 (12.7%) of 220 NR of F3. One (14.3%) of seven TR was F4 and six (19.4%) of 31 NR were F4. No HCC was noted in F1. The detection time of HCC in these 3 groups after IFN treatment was as follows: 22.7±6.5 months in SR, 22.0±7.7 months in TR, and 34.4±12.9 months in NR, respectively. The annual incidence of HCC was as follows: in F2: 0% in SR, 0.2% in TR, and 0.9% in NR; in F3: 1.2% in SR, 2.8% in TR, and 3.6% in NR; and in F4: 0% in SR, 3.7% in TR, and 5.1% in NR (Table 5). The cumulative incidence of HCC was significantly (p=0.0190) lower for all SR patients in F2 compared with NR, but not for SR compared with NR in F3 (Table 5). No significant differences were noted for TR *vs* NR in F2 and F3 or SR *vs* NR in F4; however, the cumulative incidence of HCC was significantly lower in all SR (p=0.0001) and TR (p=0.0397) compared with all NR (Fig. 1). In control F4, HCC was noted in 22 patients during their follow-up periods and the annual incidence of HCC was 6.9 %; 6.5% in the male patients <60-year-old and 11.9% in ≧60-year-old patients, respectively. In females, it was 3.8% in <60-year-old patients and 7.7% in ≧60-year-old patients. The annual incidence of HCC in patients over 60 years old was double that in patients <60 years old; however, no significant difference was noted between sexes. No significant difference was noted either in the cumulative incidence of HCC between F4 and control F4.

The ages at which patients who received IFN developed HCC ranged from 47 to 68 years (mean±SD: 61.1±6.0 years), and their mean ages were 59.0±8.1 years in F2, 61.2±5.6 years in F3, and 63.3±4.5 years in F4. Thirty-eight were male and 14 were female. Their mean ages were 61.1±5.9 years (males) and

TABLE 5

Annual and cumulative incidences of hepatocellular carcinoma (HCC) in each stage of chronic liver disease type C after interferon (IFN) therapy

Stage of liver disease	Effect of IFN therapy	No. of HCC	Annual incidence of HCC (%)	p-value[1]
F1 (n=117)	SR (n=47)	0	0	
	TR (n=39)	0	0	
	NR (n=31)	0	0	
F2 (n=636)	SR (n=194)	0	0	0.019
	TR (n=156)	1	0.2	0.168
	NR (n=286)	8	0.9	
F3 (n=355)	SR (n=73)	3	1.2	0.106
	TR (n=62)	6	2.8	0.726
	NR (n=220)	27	3.6	
F4 (n=40)	SR (n=2)	0	0	0.5479
	TR (n=7)	1	3.7	0.8031
	NR (n=31)	6	5.1	
Control F4 (n=55)		22	6.9	

p-value[1]: statistical analysis of cumulative incidence of HCC between SR and NR, and TR and NR in each stage of liver disease estimated by log-rank test.

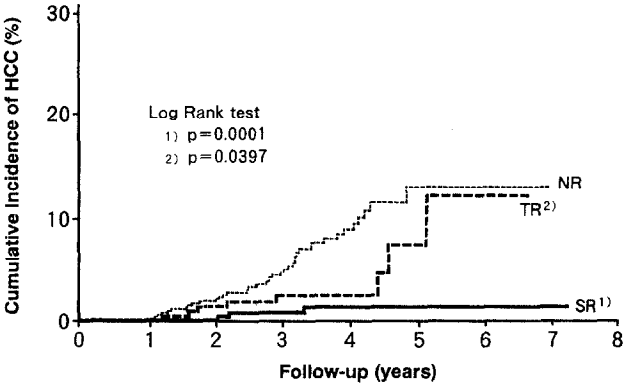

Fig. 1. Cumulative incidence of hepatocellular carcinoma in chronic hepatitis and cirrhotic type C patients who received interferon. HCC was found significantly less often in SR (p=0.0001) and TR (p=0.0397) compared with NR.

TABLE 6

Risk factors for the development of hepatocellular carcinoma after interferon therapy analyzed by basic multivariate model of the variables of sex, age, stage of liver disease, serum ALT, and PLT count

Variables	*p*-value	Conditional risk ratio and 95% confidence interval
Sex (male *vs* female)	0.0257	0.459 (0.231–0.910)
Age (years)	0.0028	1.064 (1.022–1.109)
Stage of liver disease		
F2 *vs* F3	0.0067	3.102 (1.369–7.029)
F2 *vs* F4	0.0373	3.522 (1.077–11.523)
Serum ALT	0.7655	0.999 (0.994–1.004)
PLT count	0.0815	0.929 (0.855–1.009)

Statistical analysis was performed using the log-rank test.

61.1±6.6 years (females). The mean ages of the 3 response groups were as follows: 67.0±3.0 years in SR, 63.3±4.5 years in TR, and 60.3±6.1 years in NR. There were no significant differences in the age at which HCC developed between males and females and among SR, TR, and NR. The incidence of HCC was significantly (p=0.002) higher in males than females.

Cox proportional hazard analysis of risk factors for HCC in all patients was performed with the 5 variables mentioned above. Age and stage of liver disease were extracted as independent risk factors in the development of HCC (Table 6).

The size of HCC ranged from 8 to 45 mm in diameter (mean±SD: 19.4±9.6 mm, in Table 4). In control F4, ages at detection of HCC ranged from 55 to 74 years (mean±SD; 65.6±4.7 years in all patients, 65.8±4.3 years in male and 64.9±5.8 years in female patients); the size of HCC was 25.1±10.8 mm.

Serum AFP levels at detection of HCC ranged from 1 to 824 ng/ml in the 52 HCC patients and 12 to 398 ng/ml (mean±SD: 122±139 ng/ml)in control F4 (Table 4). The average serum AFP level of the 52 HCC cases was 75±167 (mean±SD) ng/ml; however, only eight cases showed more than 100 ng/ml AFP at detection of HCC.

During the follow-up period in 1148 patients, three patients died of cancer (gastric cancer, breast cancer, and pancreas cancer). One patient died of brain tumor, one patient died of myocardial infarction and another patient died as a result of a traffic accident.

Discussion

We analyzed the effects of IFN therapy for CH-C and cirrhosis on the incidence of subsequent HCC. Development of HCC was significantly reduced in both sustained (SR) and transient responders (TR). The present study also demonstrated that PLT counts, reflecting the stage of chronic hepatitis, were significantly improved in SR after IFN therapy.

The annual incidence of HCC has been reported to be 1.4% in moderate-staged chronic hepatitis, 3% in advanced-staged chronic hepatitis, and 5 to 7% in cirrhotic type C patients in Japan (4,10,11) which is around double that in southern Europe (12,13). In the present study, the annual incidence of HCC was 0.9% in non-responders (NR) in F2, 3.6% in NR in F3, and 5.1% in NR in F4. Thus, the stage of liver disease is an important factor in the development of HCC in chronic liver disease type C. The present data also demonstrated that the age of the patients was an important factor in the development of HCC in CH-C patients. However, sex was not an independent risk factor in the present study. This might be due to the larger number of female than male F4 patients.

The causal mechanisms for development of HCC are unknown in patients with hepatitis C virus (HCV) infection. Recent experimental study in transgenic mouse using HCV core protein demonstrated that HCV itself was directly involved in the development of HCC (14), but HCC is mainly detected in patients with advanced stages of chronic hepatitis or cirrhosis and rarely seen in asymptomatic HCV carriers. Tarao et al. (15,16) reported that hepatocellular proliferation is a risk factor for the development of HCC. Maintenance of serum transaminases at low levels may protect against the development of HCC. Hepatocyte necrosis, cell damage, and increases in hepatocyte replication result in increased DNA damage, influencing hepatocarcinogenesis. Thus, development of HCC may be delayed or prevented in SR and TR.

Another possible mechanism for prevention of HCC is the direct or indirect effects of IFN. IFN has many immunologic and biologic actions, such as inhibition of cell division via transcription factor, interferon regulatory factor (IRF) (17–19), growth inhibitory actions through change in signal transduction (17,20,21), and activation of natural killer cells (22) and T-cell activity (23), resulting in tumor suppression (23–25). Lai et al. (25) reported that IFN treatment of patients with advanced HCC improved survival rates. Thus, it is possible that direct effects of IFN are responsible for the low incidence of HCC in TR and NR.

HCC was mainly detected in NR with advanced-stage chronic hepatitis or cirrhosis. These present results agree with the report by Kasahara et al. (26), who demonstrated that the risk factors for HCC and for its incidence after IFN therapy in CH-C patients were present in NR, older patients and males. They also reported that the degree of fibrosis was not a significant risk factor for the development of HCC; however, in our present study the stage of liver disease was an independent risk factor for the development of HCC.

The cumulative incidence of HCC was significantly lower in SR than in NR in F2, but was not significantly lower for TR *vs* NR in F2 and SR *vs* NR in F3 patients in the present study. PLT counts appear to reflect the stage of chronic hepatitis and the increase in PLT counts in SR of F2 and F3, and suggests improvement in the stage of liver disease after IFN therapy.

The growth rate (doubling time) of HCC is variable (26–30), ranging from 1 to 22 months (average 4 to 6 months). Sheu et al. (27) reported that the median detectable subclinical period for HCC was 3.2 years. Nevertheless, well-differentiated and hypovascular HCC have been reported to have a significantly longer doubling time than hypervascular HCC (30). Our study demonstrated that the doubling time of HCC which were 2 to 5 cm in diameter was around 3 months (unpublished data). It may take more than 6 years until HCC of diameter around 1 cm are detected by US or CT, when the doubling time of HCC is assumed to be 90 days. The volume of one HCC cell is described by the following equation, when we assume that the volume of an HCC cell is equal to that of a normal hepatocyte. The average diameter of a normal hepatocyte is considered to be 35 μm. Thus, the volume (V1) of one hepatocyte is estimated as follows:

$$V1=3/4\times3.14\times(17.5)^3\fallingdotseq1.26\times10^4\ \mu m^3.$$

The volume of HCC 1 cm in diameter is expressed as follows:

$$3/4\times3.14\ (5000)^3\fallingdotseq2.9\times10^{11}\ \mu m^3.$$

The next equation can be used when we assume that one HCC cell might grow logarithmically to the volume of HCC which are 1 cm in diameter. The doubling time $(DT)=(t_2-t_1)\ \ln2/\ln V_2-\ln V_1$ $V1=1.26\times10^4$ (μm^3), $V2=2.94\times10^{11}$ (μm^3), DT=90 days.

Using these data in the above equation, we can then get the following result:

$$t_2-t_1=2208 \text{ days (around 6.0 years).}$$

In the process of the development of HCC, many factors may affect the growth of HCC, including apoptosis and other immunological effects, possibly resulting in slower growth of HCC. Therefore, it is reasonable to consider that most HCC cases detected in the present follow-up periods had developed prior to interferon therapy, and that the growth rate of HCC was delayed by IFN in sensitive cases.

Nishiguchi et al. (31) reported that IFN reduced the development of HCC in HCV-infected cirrhotic patients, but, some questions were raised about this result (32,33). This study (31) included very small numbers of subjects and noted a marked reduction in HCC, even though SR comprised only 16% of the subjects who received IFN. This SR rate is similar to that in F3 stages in our study. A European group (34) reported that the response to interferon-alpha with biochemical resolution for cirrhosis type C was 9% and that IFN therapy did not significantly diminish the rate of the development of HCC compared with cirrhotic patients without IFN therapy, supporting our present data. However, a recent retrospective cohort study demonstrated that IFN-alpha lowered the rate of progression of cirrhosis to HCC (35).

In the present study, there were only 40 cirrhotic patients (F4), and two cases (5.0%) were SR. The annual incidence of HCC was 5.1% NR in F4, which was influenced by the patients' age at entry, as demonstrated in this study. Furthermore, no significant differences were noted in the cumulative incidence of HCC among SR, TR, and NR patients with advanced liver disease (F3 and F4). The discrepancy between our results and those of the international study group (35) might be due to the difference in patients' backgrounds, because the annual rate of development of HCC in cirrhotic type C patients in Japan was double that in European countries. Especially, in Japanese cirrhotic patients a high frequency of undetectable-sized HCC had already developed before IFN treatment, resulting in the failure of inhibition of HCC by IFN therapy. Further studies are needed to define the effects of IFN on the development of HCC in advanced-stage hepatitis C patients.

In conclusion, IFN therapy for chronic hepatitis C patients resulted in 27.5% sustained responders, 23.0% transient responders, and 49.5% non-responders. Development of HCC was significantly reduced or delayed in both sustained and transient responders with CH-C, but not significantly in advanced-stage patients.

Appendix

In addition to the study authors, the investigators in the Viral Hepatitis Therapy Study Group included: M. Miyoshi, Department of Internal Medicine, Kyoto First Red Cross Hospital; S. Takamori, Second Department of Internal Medicine, Matsushita Memorial Hospital; T. Ogasawara, and T. Nakajima, Center of Digestive Disease, Ohtsu Municipal Hospital; K. Kagawa, Department of Internal Medicine, Fukuchiyama City Hospital; Y. Katsuma, Department of Internal Medicine, Kyoto Municipal Hospital; M. Takeda, Department of Internal Medicine, Rakuwakai-Marutamachi Hospital; Y. Nakagawa and H. Tada, Department of Digestive Disease, Hoshigaoka-Kohseinenkin Hospital; M. Ohta, Department of Internal Medicine, Saiseikai Kyotofu Hospital; Y. Sawa, Department of

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