ment, compared to surgical treatment. It can be performed under local anesthesia when a tumor nodule is located at a visible and feasible site in the liver. The treatment produces a necrotic area of 30–35 mm in diameter depending on varied RFA devices and apparatus, which is usually larger than PEI or MCT. RFA therapy, therefore, produces better results than PEI or MCT in the ablation of HCC without treatment repetition. Transient arterial obliteration or other devices proved to increase the size of ablation area in RFA therapy [19,20]. Since more than 80% of patients with HCC are associated with cirrhosis, less invasiveness is inevitable condition for a safe treatment. RFA also proved to be inexpensive compared to surgery (¥867,200 versus ¥1,745,100), in the treatment of a small HCC. Although RFA is believed to be cost-effective and suitable for management of HCC of 3 cm or less in diameter, it sometimes brings about local recurrence of the tumor at the site of ablation. We, therefore, analyzed the cost-utility of the treatment.

RFA showed a higher local recurrence rate after therapy than surgical therapy (7.9% versus 0% at the end of the second year). Although local recurrence after tumor ablation was sometimes associated with invasive characteristic or intrahepatic tumor dissemination, our patients did not show such a malignant feature at the time of recurrence. Regular check-up with CT detected the recurrent tumors as a small-sized HCC of 2 cm or less, and immediate application of loco-regional therapy was performed in every patient. Indeed multicentric tumor recurrence and intra-hepatic metastasis did occur in both treatment groups, a total of 10 patients had to undergo an additional ablation therapy because of "insufficient initial treatment" in the RFA therapy group. Since all but one patient with local tumor recurrence had grade 2 necrosis at the end of initial session of RFA therapy, an achievement of grade 3 necrosis proved very important as a chief aim of the therapy. Interestingly, when additional ablation therapy was performed for patients with HCC of grade 1 or 2, local recurrence rate significantly decreased even in those patients with eventual necrosis grade 2 after therapy repetition. The reason why local recurrence rate was reduced in the "same" grade 2 in the repeated therapy group was because invisible and unidentifiable small tumors adjacent to major vessels were finally ablated by supplementary treatment. If a tumor nodule is located adjacent to major vessels such as portal vein or hepatic vein, it cannot be ablated with a good necrosis margin of grade 3 even after repeated therapy. Current data shows that supplementary treatment for those tumors with grade 2 necrosis is very important even when it is not considered to become grade 3 necrosis for the reason of the surrounding vascular condition.

Among 52 patients with multiple ablation therapy for insufficient necrosis of grade 1 or 2, 31 patients (59.6%) achieved grades 3 and 21 patients (40.4%) remained at grade 2. Local recurrence rates after therapy repetition in grades 3 and 2 were 0% and 10.3% at the end of the second year, respectively. If all the patients with grades 1 and 2 necrosis after initial RFA therapy underwent additional procedures to achieve grade 3 necrosis, simulated rates of grades 2 and 3 were 24.0% and 76.0% after therapy iteration. From these figures of the simulation, cumulative local recurrence rate was estimated as 3.3% at the end of the second year. Additional tumor ablation proved to be of importance in the management of a small HCC.

Cost-effectiveness was compared between surgery and RFA, taking into account that RFA therapy is associated with a small percentage of local recurrence. Net costs of treatment practice for RFA therapy (single procedure), RFA therapy (twice procedures), and surgery were ¥143,600, ¥243,600, and ¥438,200, respectively. The period of hospital stay was significantly shorter in patients with RFA therapy than in patients with surgery, and the basic charge for admission was directly associated with the length of hospital stay. When RFA was chosen as treatment for a small HCC, total medical expense for twice the procedures was much less than surgery by ¥659,100 per person. Since RFA therapy is accompanied by 7.9% of local recurrence as a whole, an additional cost of ¥100,400 per person for treatment of recurrent tumors is required from the viewpoint of social expense. Finally, RFA was more cost-effective by as much as ¥741,600 per person, in the treatment of a small HCC of 3 cm or less in diameter.

Although this study emphasized the cost-effectiveness of RFA therapy, surgical therapy was indispensable for the management of a small HCC of 3 cm or less in diameter. Good indications for surgery include a superficial tumor, a tumor facing to the intestines or gall bladder, a tumor located in the caudate lobe, a US-invisible tumor, or a tumor surrounded by major portal or hepatic vein. In this study, we analyzed a retrospective cohort of patients with HCC, as many as 57 (35.6%) of 160 patients with a small HCC development for the first time received surgical resection. Recent advances in surgical procedures and instruments seldom cause morbidity and mortality in the treatment of a small HCC, and a blood transfusion is usually not necessary during and after resection. Surgery should, therefore, be applied complementarily to patients with a small HCC, depending on the location of the tumor, liver function, and background features of the host.

In conclusion, RFA was superior to surgical resection if a small HCC can be managed with percutaneous procedures, from the viewpoint of cost-effectiveness. It is also emphasized that an additional ablation therapy for patients with grade 1 or 2 necrosis after initial RFA significantly reduced the risk of local recurrence. Questionnaires about quality of life for the patients with RFA and surgical resection should be also analyzed to compare the superiority of the two treatment arms.

Acknowledgement

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Hepatitis B Virus-Related Hepatocellular Carcinogenesis and Its Prevention

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

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

Key Words
Carcinogenesis · Hepatocellular carcinoma · Liver cirrhosis · Hepatitis B virus · DNA · Interferon · Cancer prevention

Abstract
To elucidate the influence of serum hepatitis B virus (HBV) load on hepatocellular carcinogenesis in cirrhotic patients, HBV-DNA was sequentially measured. In a nested, case-control study using 96 patients without antiviral therapy, high HBV-DNA ($\geq 10^{3.7}$ copies/ml) in the last 3 years was significantly associated with carcinogenesis (a patient group without hepatocellular carcinoma (HCC) development; 0/48 vs. a patient group with eventual HCC development; 22/48, $p < 0.0001$). No patient with a continuously low HBV-DNA for the last 3 years developed HCC. Persistence of high HBV-DNA concentration suggested an increased risk of carcinogenesis. In a retrospective cohort study using 57 patients with interferon therapy, HCC developed in 2 (8.0%) of the 25 patients with HBV-DNA loss, while carcinogenesis was found in 11 (34.4%) of 32 patients without HBV-DNA loss (Fisher's exact test, $p = 0.026$). A significant decrease or loss of serum HBV-DNA stops HCC development, and its sequential analysis could be very useful both in the prediction and early detection of small HCC.

Introduction

Hepatocellular carcinoma (HCC) is a principal cause of death in many parts of sub-Saharan Africa and in Asia [1, 2]. It is also one of the most common neoplasms in Japan [3]. Abundant epidemiological and molecular biological evidence shows that hepatitis B virus (HBV) is an important factor in the development of HCC [4–6], but the precise role of HBV DNA viruses in oncogenesis is still unknown. Although increasing evidence indicates that the HBV plays an important role in the development of HCC after discovery of integrated forms of HBV [7–9], current serological and virological markers are still insufficient in establishing this relationship. Since a really curative therapy is not available for HCC at present, an accurate prediction and early detection of HBV-related HCC is essential in the current situation.

Hepatocellular carcinogenesis rates were estimated in patients with HBV-related chronic hepatitis (n = 297) and cirrhosis (n = 246), who have not received interferon (IFN), lamivudine, or steroid therapy. They were diagnosed by peritoneoscopy and/or biopsy as having chronic liver disease in the Toranomon Hospital, Tokyo, Japan, from 1974 to 1999. Cumulative carcinogenesis rates in F1 fibrosis, F2–3, and F4 were 0.5, 6.3, and 19.7% at the end of the 5th year, 2.7, 14.9, and 30.3% at the end of the 10th year, 4.1, 19.5, and 35.8% at the15th year, and 15.0, 29.6, 41.9% at the 20th year, respectively (fig. 1).

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Kenji Ikeda, MD
Department of Gastroenterology, Toranomon Hospital
Toranomon 2-2-2, Minato-ku
Tokyo, 105-8470 (Japan)
Tel. +81 44 877 5111, Fax +81 44 860 1623, E-Mail ikedakenji@tora.email.ne.jp

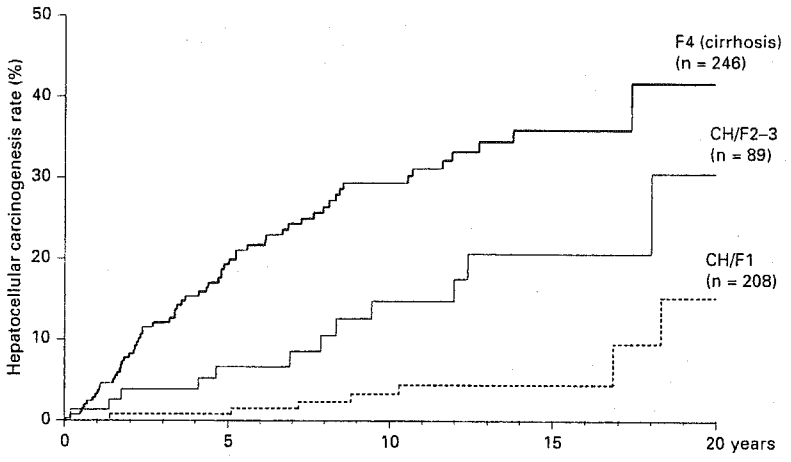

Fig. 1. Cumulative hepatocellular carcinogenesis rates in patients with chronic hepatitis or cirrhosis.

Needless to say, patients with HBV-related cirrhosis have a significantly higher risk for HCC development [10, 11], but the degree of the carcinogenesis risk in an individual patient cannot be predicted as yet. How can we recognize a super-high-risk group or a rather low-risk group in HBV-related cirrhosis? Can we predict and specify a patient who is not likely to develop HCC in the future? Hepatocellular carcinogenesis in patients with HBV infection may well be associated with persistence of aminotransferase, concentration of HBV DNA, or merely the severity of the liver disease. One of the purposes of this article is, therefore, to elucidate the relationship of hepatocellular carcinogenesis with longitudinal clinical courses of biochemical data and HBV DNA concentration in consecutive patients with cirrhosis.

IFN has been reported to be effective in patients with HBV-related chronic hepatitis, which decreases serum HBV DNA concentration and improves biochemical data on early control studies [12–14], and subsequently suppresses disease progression to cirrhosis [15, 16]. Although various effects of IFN in hepatitis B virus infection have been well investigated from virological, biochemical, and medico-economical viewpoints [17–19], the influence on a long-term outcome of liver cirrhosis or on hepatocellular carcinogenesis is still controversial [20–25]. In order to clarify the mechanism of anti-carcinogenic activity of IFN, if any, we analyzed HBV DNA concentration serially in a cohort of 60 patients with cirrhosis. The other purposes of this study are to elucidate the relationship of hepatocellular carcinogenesis with longitudinal clinical courses in consecutive cirrhotic patients with interferon therapy and to investigate an early prediction of HBV DNA elimination and the cancer preventive activity.

Factors Affecting Hepatocellular Carcinogenesis in Cirrhosis (without Anti-Viral Therapy)

Patients and Methods

Analyzable Patients without Anti-Viral Therapy

Among 217 patients who were diagnosed as having HBV-related cirrhosis by peritoneoscopy and/or liver biopsy from 1976 to 1989 in our hospital, 160 patients had not undergone interferon or other antiviral therapy. Out of the consecutive 160 patients, sequential assay of serum HBV DNA using serial sera stored at −80°C was available in 146 patients (91.3%). All 146 patients showed a positive hepatitis B surface antigen and negative anti-hepatitis C virus antibody in the assay of their initial sera. Among the 146 patients with HBV-related cirrhosis, 48 patients (32.9%) developed HCC during a median follow-up period of 7.2 years after the diagnosis of cirrhosis, and the other 98 patients (67.1%) have not developed HCC during 11.7 years.

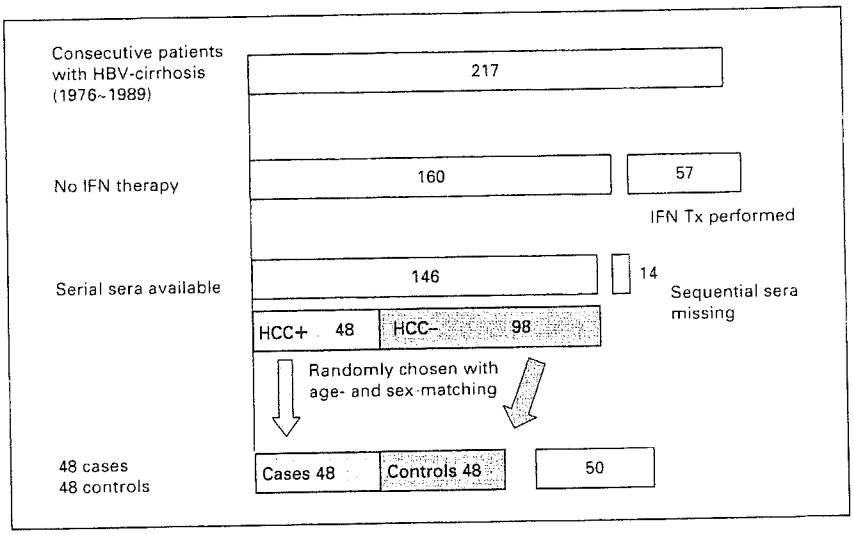

Fig. 2. Analyzed patients with HBV-related cirrhosis. Since almost all of consecutive untreated patients (146/160, 91.3%) were analyzable, a nested case-control study was established using a total of 96 patients.

Follow-Up of Patients and Diagnosis of HCC

Follow-up of the patients was made on a monthly or bi-monthly basis after diagnosis of liver cirrhosis by monitoring α-fetoprotein (AFP) and other biochemical data. Imaging diagnosis was carried out two or more times per year for each patient with computed tomography (CT), ultrasonography (US), or scintigraphy. HCC was diagnosed by typical hypervascular characteristics on angiography in addition to certain features of CT and US. A pathological confirmation of surgically resected specimens or autopsy was made in 38 (79.2%) of 48 patients with HCC development.

Nested Case-Control Study

In order to elucidate the relationship between hepatocellular carcinogenesis and longitudinal courses of clinical markers, a nested case-control study was introduced. Age- and gender-matched control patients were selected from the 98 HCC-free patients with a ratio of 1:1. The control patients were randomly selected using a computer-generated random number table, avoiding those with a short observation period of less than 3 years. Thus, a nested case-control study was made, consisting of 48 cases with cancer development (group A) and 48 demography-adjusted controls without sings of cancer (group B) (fig. 2).

Assays of HBV Markers

HBV DNA was assayed using frozen sera stored at −80°C, and quantified using transcription mediated amplification and hybridization protection assay described by Kamisango et al. [26] (TMA-HPA, Chugai Diagnostics Science, Tokyo, Japan). A lower value of HBV DNA of 3.7 LGE/ml (equivalent for $10^{3.7}$ copies/ml or 5,000 copies/ml) was considered as a low value. For annual sera from the diagnosis of cirrhosis to the end of observation period in each patient, the DNA quantification was simultaneously performed after fixation of the 48 cases and the 48 controls.

Statistical Analysis

Standard statistical measures and procedures were used. Mann-Whitney U test, χ^2 test, and Fisher's exact test were employed for examination of background characteristics of the patient groups with and without HCC development. $p < 0.05$ with the two-tailed test was considered significant. Data analysis was performed using the computer program SAS version 6.12 [27].

Results

Demography and Initial Laboratory Data of the Groups with or without HCC Development [28]

Table 1 shows the demography and initial laboratory data of the patients in groups A and B. The ratio of men was 39 of 48 (81.3%) in the both groups, and the median age was 49.5 and 49 in groups A and B, respectively. The proportion of decompensated cirrhosis, and a history of past alcohol consumption, were not significantly different

Table 1. Demography and initial laboratory data of 48 patients with HCC development and the 98 patients without HCC development during the observation period

	Group A HCC development (n = 48)	Group B no HCC (n = 48)	p
Demography			
Men:women	39:9	39:9	NS
Age, median (range)	49.5 (30–71)	49 (30–71)	NS
Decompensated cirrhosis	1 (2.1%)	7 (14.6%)	0.65
Past alcohol consumption of 500 kg or more	8 (16.6%)	9 (18.8%)	0.79
Initial laboratory data (median, range)			
Anti-HCV antibody positive	0	0	NS
HBe antigen positive	33/48 (68.8%)	17/48 (36.1%)	0.001
Bilirubin, mg/dl	1.0 (0.6–9.8)	1.0 (0.5–7.5)	0.46
Albumin, g/dl	3.95 (2.4–4.8)	4.0 (2.5–5.2)	0.23
Aspartic transaminase, IU	39.5 (15–820)	31.5 (13–376)	0.23
Alanine transaminase, IU	32 (8–740)	31 (9–313)	0.82
Platelet count, $\times 10^3/mm^3$	100 (28–225)	121 (49–255)	0.047
AFP, ng/ml	16 (3–785)	7 (3–1,520)	0.037

between the two groups. The prevalence of positive HBe antigen was, however, significantly higher in group A than that in group B. Although median platelet count was slightly lower, and alpha-fetoprotein concentration was higher in group A, there was no significant difference in bilirubin, albumin, aspartic transaminase, and ALT between the two groups.

Individual HBV DNA Concentration until the End of the Observation Period [28]

Quantitative HBV DNA assessment was sequentially performed until the diagnosis of HCC in each patient. In group A (HCC development), 9 patients showed intermittently high HBV DNA concentration and 39 patients showed a continuously high HBV DNA concentration from the diagnosis of cirrhosis to the development of HCC. All the patients experienced high HBV DNA during their clinical courses, and no patient showed low HBV DNA for a consecutive 3 years just before the detection of HCC.

Serial HBV DNA concentration of each patient was also assessed in group B (no HCC development). HBV DNA was continuously low in 9 patients, and HBV DNA concentration showed a settling down and lowering for 3 years or more until the end of observation period in 13 patients. Nine patients showed a fluctuated HBV DNA concentration, and the remaining 17 patients had a continuously high HBV DNA during the observation period. Of the 48 patients, 9 patients never experienced a high HBV DNA load, and a total of 22 patients (45.8%) showed low HBV DNA values for a successive 3 years until the end of the observation (fig. 3).

The incidences of HBV DNA patterns were significantly different between the two groups (χ^2 test, $p < 0.001$). The rates of low or a settling down trend of HBV DNA concentration was significantly lower in group A

Table 2. Demography and laboratory data of 57 patients with HBV-related cirrhosis undergoing interferon therapy

Demography	
Men:women	45:12
Age, median (range)	41 (19–60)
Decompensated cirrhosis	3 (5.3%)
Past alcohol consumption of 500 kg or more	3 (5.3%)
Laboratory data, median (range)	
Bilirubin, mg/dl	0.9 (0.4–2.6)
Albumin, g/dl	4.1 (3.0–4.9)
Aspartic transaminase, IU/l	65 (16–404)
Alanine transaminase, IU/l	74 (12–586)
Platelet count, $\times 10^3/mm^3$	125 (68–332)
Anti-HCV antibody positive	0
HBe antigen positive	41 (71.9%)
HBV-DNA, LGE/ml[1]	7.2 (3.9 to >8.7)
Observation period, years	13.6 (6.5–16.1)

[1] HBV-DNA (LGE/ml): log-genome equivalent, expressed as 10^n copies/ml.

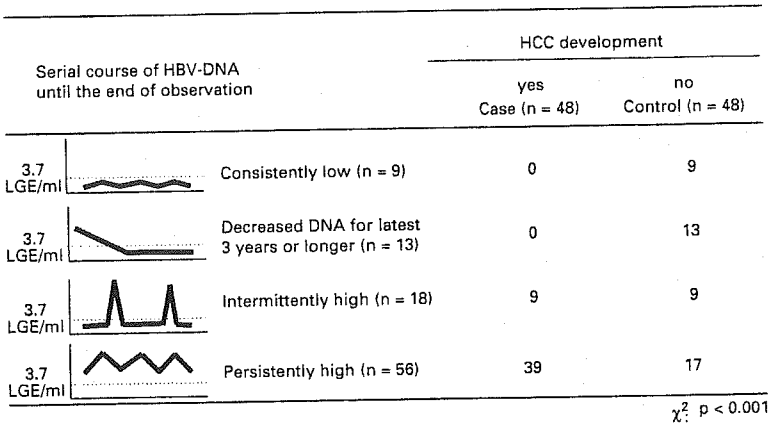

Fig. 3. Patterns of longitudinal courses of HBV-DNA in groups A and B.

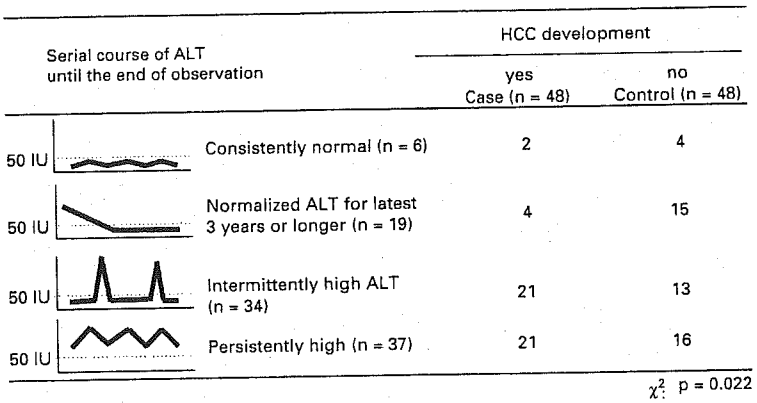

Fig. 4. Patterns of longitudinal courses of alanine transaminase in groups A and B.

than in group B (0/48 in group A vs. 22/48 in group B, Fisher's exact test, $p < 0.00001$). Any patients with a continuously low HBV DNA concentration for 3 years or longer did not develop HCC during the clinical courses.

Patterns of Longitudinal Courses of Alanine Transaminase [28]

ALT values were also assessed sequentially throughout the entire clinical courses. In group A, ALT was continuously normal in 2 patients (4.2%), ALT was high initially but normalized for the last 3 years or longer in 4 (8.3%), it showed abnormal values intermittently in 21 (43.8%), and had a continuously high value during the observation period in the remaining 21 (43.8%). In group B, 4 patients (8.3%) showed consistently normal ALT, 15 (31.3%) showed a decrease in ALT values, 13 (27.1%) intermittent elevation, and the remaining 16 (33.3%) showed continuously high ALT values during the follow-up period (fig. 4).

The incidence of HBV DNA patterns was significantly different between the two groups (χ^2 test, $p = 0.022$). While persistently or intermittently elevated ALT value slightly favored higher carcinogenesis rate (42/48 in group A vs. 29/48 in group B), statistical significance was, however, not obtained between carcinogenesis and ALT values (χ^2 test, $p = 0.077$).

Discussion

Liver cirrhosis due to hepatitis C virus usually shows a rather steady and constant clinical course, which enables us to estimate the future carcinogenesis rate from only clinical information at the time of the diagnosis of cirrhosis. Disease activity and carcinogenic potency of HBV-related liver disease, on the contrary, often change in natural clinical courses, accompanying significant fluctuation of HBe antigen system or amount of HBV DNA. When we investigate the relationship between hepatocellular carcinogenesis and its affecting and contributing factors, explanatory parameters should include not only initial demographic data but also chronological clinical data after starting the observation [29]. A longitudinal analysis is, therefore, necessary for the study of carcinogenesis in chronic liver disease caused by HBV. We, therefore, established a nested case control study using longitudinal clinical data until the end of the observation period or just before carcinogenesis, including HBV DNA quantification and ALT.

In this study, the sequential trend of serum HBV DNA concentration was significantly associated with hepatocellular carcinogenesis, and the relationship of HBV DNA to the carcinogenesis was much stronger than that of ALT. Indeed, mere initial background features and laboratory data of the patients could predict a future risk of carcinogenesis, and the chronological analysis demonstrated more discrete differentiation of a high-risk group and provided more detailed information about HBV-related carcinogenesis. Although this study illustrates that a consistence of low HBV-DNA concentration for 3 years or longer saves cirrhotic patients from carcinogenesis, the combination of 'low HBV-DNA' and '3 years' might not avoid the carcinogenesis risk sufficiently, considering the fact that hepatocellular carcinoma does develop without hepatitis, without high ALT, or without high HBV-DNA. It is, however, true that HCC scarcely develops in a patient with HBV-related cirrhosis whose HBV-DNA concentration is consistently low for the recent 3 years or longer.

Although a high load of HBV-DNA seems to promote carcinogenesis or tumor growth, the reason why a high concentration of HBV-DNA affected hepatocellular carcinogenesis remains unknown. Taking into account that hepatitis patients with positive HBe antigen and fluctuated aminotransferase values often show a high serum HBV-DNA concentration, a large amount of HBV-DNA load may be associated with a high carcinogenesis rate through an active inflammatory state and indirect cancer promotion [30]. Relationship between hosts and hepatitis virus should also be considered in future studies on carcinogenesis.

Hepatocellular Carcinogenesis in Cirrhotic Patients with Interferon Therapy

Patients and Methods

Analyzed Patients

Among 189 patients who were diagnosed as having HBV-related cirrhosis by peritoneoscopy and/or liver biopsy from 1983 to 1990 in our hospital, a total of 60 patients underwent interferon therapy from 1986 to 1990. Since 3 patients were lost to follow-up, the remaining 57 patients (95.0%) were analyzed for virological outcome, carcinogenesis, and eventual prognosis: the reason for the dropout from the observation in the 3 patients was simply house moving.

Interferon Therapy

IFN-α was administered in 35 patients (61.4%) and IFN-β in the remaining 22 patients (38.6%). The daily amount of IFN was 3 million units in 22 (38.6%) and 6 million units in 35 (61.4%), and twice a week administration was performed in 54 (94.7%) and three times a week in 3 (5.3%). All patients received an intermittent interferon therapy for a median of 18 months (range 2–132 months), but the duration of the IFN therapy was arbitrary in this pilot study. Although the amount of daily dose of IFN and the duration of the therapy varied in this study, 52 (91.2%) of the 57 patients received IFN for 6 months or longer.

Follow-Up and Diagnosis of HCC

Follow-up of the patients was made on a monthly basis after diagnosis of liver cirrhosis by monitoring virological, hematological, and biochemical data including α-fetoprotein (AFP). All these laboratory tests including HBV-markers were obtained throughout the observation period in each patient. Patients were classified into four groups according to patterns of serial concentration of HBV DNA: type A, disappearance of HBV DNA during and after IFN therapy; type B, loss of HBV DNA after cessation of IFN administration; type C, transient loss of HBV DNA only during IFN administration; type D, persistently positive HBV DNA during and after the therapy. Clinical courses of ALT fluctuation were also classified into four groups according to normalization of ALT value.

Imaging diagnosis and establishment of diagnosis of HCC were carried out as shown above.

Assay of HBV DNA

HBV-DNA was assayed using frozen sera stored at -80°C, and quantified using transcription mediated amplification and hybridization protection assay described by Kamisango et al. [26] as shown above.

Statistical Analysis

Standard statistical measures and procedures were used. Mann-Whitney's U and χ^2 tests were employed for examination of background characteristics between the groups with and without HBV DNA elimination. Fisher's exact test was also used to analyze the relationship of HBV markers with carcinogenesis. Cumulative HBV DNA disappearance rate, carcinogenesis rate, and survival rate were calculated by Kaplan-Meier technique [31], and the differences between the analyzed groups were assessed by log-rank test. $p < 0.05$ with the two-tailed test was considered to be significant. Data analysis was performed using the computer program SPSS version 11 [32].

Results

HBV DNA in Clinical Courses [33]

HBV DNA was positive in all the patients at the initiation of IFN therapy (3.9 to >8.7 LGE/ml). HBV DNA became negative (<3.7 LGE/ml) in 25 of 57 patients (43.9%) during the observation period with a median of 13.6 years. The remaining 32 patients did not show a sustained negative HBV DNA after the therapy, although 9 patients did show transient negative values for a limited period during the therapy.

Clinical courses of HBV DNA were classified into the four categories mentioned above. Nine patients (15.8%) lost HBV DNA during and after IFN therapy (type A), 16 patients (28.1%) lost HBV DNA after cessation of the therapy (type B). The other 9 patients (15.8%) showed a transient loss of HBV DNA (type C), and the remaining 23 (40.4%) retained persistently positive HBV DNA (type D).

Cumulative rate of HBV DNA disappearance was calculated using Kaplan-Meier technique. DNA became negative in 10.5% at the end of the first year after initiation of IFN, 12.3% at the third year, 21.0% at the fifth year, 43.7% at the tenth year, and 46.7% at the fifteenth year, respectively.

Hepatocellular Carcinogenesis and Serial Concentration of HBV DNA [33]

A total of 13 patients developed HCC during the observation period.

The relationship between carcinogenesis and serial concentration of HBV DNA was analyzed (fig. 5). No patients (0%) developed HCC among 9 patients in type A. Two (12.5%) of 16 patients developed HCC in type B: HCC were detected 1.2 year after disappearance of HBV DNA in one patient, and 3.6 years after disappearance of HBV DNA in the other patient. Three (33.3%) of 9 patients showed carcinogenesis in type C, and 8 (34.8%) of 23 patients developed HCC in type D during the observation. Hepatocellular carcinogenesis was significantly associated with persistent positive HBV DNA after initiation of IFN (2/25 vs. 11/32, $p = 0.019$ by χ^2 test, $p = 0.026$ by Fisher's exact test).

Cumulative carcinogenesis rates were analyzed according to the ultimate courses of serial assay of HBV DNA. Fifth-year hepatocellular carcinogenesis rate were 0% in patients with HBV DNA loss, and 9.4% in patients without HBV DNA elimination, 10-years rates were 8.0 and 22.5%, and 15-year rates were 8 and 44.0%, respectively. The carcinogenesis rate in patients with HBV DNA elimination was significantly lower than those without DNA elimination ($p = 0.011$, log-rank test).

Hepatocellular Carcinogenesis and HBe Antigen and Aminotransferase [33]

Relationship was assessed between carcinogenesis and HBeAg positivity during the clinical courses. HBeAg was positive in 41 patients (71.9%) and negative in 16 (28.1%) at the initiation of IFN therapy. Twenty-eight (68.3%) of the 41 patients showed continuous loss of HBeAg after IFN therapy. HCC developed in 4 (25.0%) of the 16 patients without HBeAg from the beginning. 4 (14.3%) of the 28 patients with HBeAg clearance, and 5 (38.5%) of 13 patients with persistent HBeAg positivity. HBeAg clearance did not significantly decrease the incidence of carcinogenesis risk ($p = 0.12$, χ^2 test with Yates' correction).

Relationship was also analyzed between carcinogenesis and a longitudinal course of ALT after IFN therapy. Four (18.2%) of 22 patients with normalization of ALT after IFN therapy developed HCC, 9 (25.8%) of 35 patients with persistent abnormal ALT developed HCC. Serial values of ALT was not significantly associated with carcinogenesis risk ($p = 0.075$, χ^2 test with Yates correction).

Cumulative HBe antigen disappearance rate, HBV-DNA disappearance rate, and ALT normalization rate

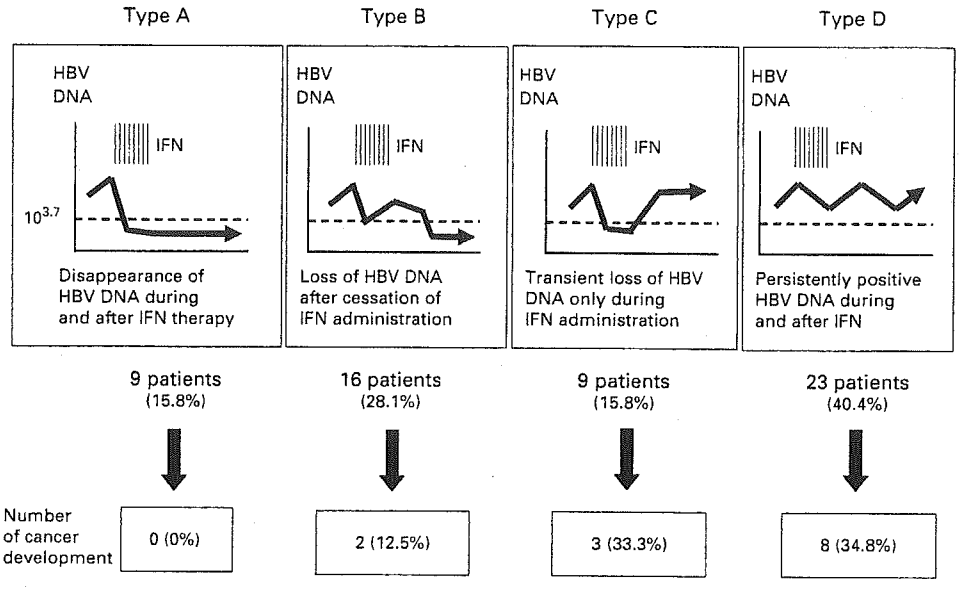

Fig. 5. Relationship between types of serial HBV-DNA concentration and carcinogenesis.

were calculated in those patients with positive HBe antigen at the beginning of IFN treatment. HBe antigen disappearance rate and DNA disappearance rate were 55.4 and 14.6% at the end of the 5th year, and 55.4 and 40.1% at the 10th year, respectively. ALT normalization rate at the 5th year was 25.4% and 10th-year rate was 41.2%. Although the incidence of virological and biochemical improvement gradually increased after the therapy, the rates evidently differed among them.

Discussion

Until recently, several authors mentioned the anti-carcinogenic activity of IFN in patients with HBV-related cirrhosis. Oon [20] and we [23] showed that IFN significantly decreased a carcinogenesis in patients with IFN therapy with a relative risk of 0.03 and 0.39, respectively. Lin et al. [25] also demonstrated an anti-tumor activity of IFN with a relative risk of 0.11 in a randomized controlled trial for patients with chronic hepatitis and cirrhosis. Mazzella et al. [21], Fattovich et al. [22], and the International Interferon-alpha Hepatocellular Carcinoma Study Group in Europe [24] demonstrated a low relative risk for carcinogenesis in patients with IFN therapy, but they could not show a statistical significance. Aside from the slightly inconsistent results after IFN therapy for cirrhosis, we tried to elucidate the relationship between virological response and HCC development, using a cohort of consecutive patients with cirrhosis who underwent IFN therapy more than 10 years ago. Considering that the disease activity and carcinogenic potency can change significantly in the course of HBV-related liver disease, a longitudinal analysis was performed for the study of clinical process and mechanism of anti-tumor activity of IFN in HBV-positive cirrhosis.

In this clinical study, sequential trends of HBV concentration were significantly associated with hepatocellular carcinogenesis, as was found in natural clinical courses of patients without IFN [28]. Although only 2 of 25 patients developed HCC who showed a disappearance of HBV-DNA during or after IFN therapy, 11 of 32 patients showed carcinogenesis who could not eliminate HBV DNA by the treatment with IFN ($p = 0.019$). Hepatocellular carcinogenesis was assessed using serial HBV DNA assay with a cut off value of 3.7 LGE/ml or $10^{3.7}$ copies/ml in this study. Although a detailed analysis of HBV-DNA concentration with more sensitive measurement may

demonstrate a better correlation with carcinogenesis rate than current one, this setting of HBV-DNA concentration as a cut-off value was significantly valuable in the prediction for HCC appearance.

Although the mere use of IFN does not guarantee the decrease of carcinogenesis in patients with HBV-related cirrhosis, a serial course of HBV DNA concentration was significantly correlated with the future HCC development during and after treatment. The value of cancer prediction was much higher in the assay of HBV DNA than that of HBe antigen. Indeed the cut-off values of HBV DNA concentration seemed to be discretionary, the advantage in clinical practice was marked and conspicuous. When more sensitive ways of HBV DNA concentration were applied to the analysis, hepatocellular carcinogenesis could be more successfully predicted.

Conclusions

Persistence of high concentration of HBV DNA was significantly associated with hepatocellular carcinogenesis in cirrhotic patients with and without IFN therapy and its sequential analysis would be useful in early detection of HCC. Further studies with a greater number of patients are required to confirm the relationship, and future studies should be aimed at defining the basic mechanism of hepatocellular carcinogenesis and the role of IFN by which the carcinogenesis rate was suppressed in the cohort.

Acknowledgment

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Anticarcinogenic Impact of Interferon on Patients with Chronic Hepatitis C: A Large-Scale Long-Term Study in a Single Center

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

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

Key Words
Cirrhosis · Fibrosis · Hepatitis C virus · Hepatocellular carcinoma · Interferon

Abstract
Background: The anticarcinogenic capacity of interferon (IFN) was assessed in a cohort of Japanese patients with chronic hepatitis C en masse. ***Patients and Methods:*** The rate of hepatocarcinogenesis was analyzed in 2,166 patients with chronic hepatitis C, of whom 1,654 had received IFN therapy while 512 had not. ***Results:*** Crude rates of hepatocarcinogenesis in treated and untreated patients were 2.6 and 4.6% at the end of the 5th year, 5.8 and 12.7% at the 10th year and 13.9 and 23.9% at the 15th year (after completion of IFN therapy for those treated) ($p < 0.001$). IFN decreased the hazard ratio of carcinogenesis to 0.42 ($p < 0.001$) in multivariate analysis with adjustments for significant covariates including fibrotic stage, γ-glutamyl transpeptidase level, gender, platelet count and age. Among the 1,654 patients treated with IFN, 606 (36.6%) achieved persistent loss of hepatitis C virus (HCV) RNA and an additional 266 (16.1%) gained normal levels of alanine aminotransferase without loss of HCV RNA for 6 months or longer after the completion of IFN therpay. Cumulative rates of hepatocarcinogenesis in sustained virological responders and biochemical responders were 1.4 and 2.0% at the end of the 5th year, 1.9 and 3.6% at the 10th year and 1.9 and 7.5% at the 15th year, respectively. The hazard ratio of sustained virological response was 0.10 ($p < 0.001$), and that of biochemical response was 0.12 ($p < 0.001$). Normalization of aminotransferase levels after IFN therapy without loss of serum HCV RNA decreased hepatocarcinogenesis. ***Conclusion:*** IFN significantly decreased the rate of hepatocarcinogenesis in patients with chronic hepatitis C as a whole in Japan, even in those who fail to clear HCV RNA from serum.

Introduction

In most developed countries, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for the great majority of hepatocellular carcinoma (HCC), with incidence rates dependent on the regional prevalence of these hepatitis viruses. HCV-associated HCC typically develops through a sequence of events that progress from chronic inflammation through fibrosis and cirrhosis accompanying dysplasia and ultimately to HCC. In our previous cohort study on Japanese patients with HCV-related cirrhosis [1], cumulative rates of developing HCC at 5, 10 and 15 years were 21.5, 53.2 and 75.2%, respectively. According to our observations of untreated patients with chronic hepatitis C [2], rates of hepatocarcino-

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Kenji Ikeda, MD
Department of Gastroenterology, Toranomon Hospital
Toranomon 2-2-2, Minato-ku
Tokyo 105-8470 (Japan)
Tel. +81 44 877 5111, Fax +81 44 860 1623, E-Mail ikedakenji@tora.email.ne.jp

genesis at 5, 10 and 15 years were estimated to be 4.8, 13.6 and 26.0%, respectively. The life expectancy of patients with HCV-related cirrhosis is largely influenced by the development of HCC in the clinical course. As the efficacy of radically curative therapies for HCC remains limited at best, and since a severe organ shortage does not provide with sufficient chances for liver transplantation, the prevention of HCC in patients with chronic liver disease is of great importance at the present.

Interferon (IFN) is effective in eliminating HCV and reducing serum levels of alanine aminotransferase (ALT) in some patients with chronic hepatitis C [3–6]. Reduced incidence of HCC in HCV-associated cirrhosis by IFN has been reported by many investigators including ourselves [7–14]; only a few studies have failed to find its benefit [15, 16]. However, many published studies had shortcomings in the study design, in terms of pooling patients who received IFN in diverse regimens, relatively short periods of follow-up despite a long incubation period of HCC, large numbers of dropouts and retrospective studies with historical controls. Moreover, almost all studies evaluated the activity of IFN to prevent HCC by comparing responders and nonresponders to the treatment. Due to difficulties in studying patients with chronic hepatitis C, a number of nonrandomized studies examined the effect of IFN on the incidence of hepatocarcinogenesis [17–20]. With invariable limitations in study design and interpretation of the results, these studies have disclosed useful information as regards the treatment of patients with chronic HCV infection.

In order to evaluate whether IFN can reduce the rate of carcinogenesis in patients with chronic hepatitis C, we compared 1,654 patients with IFN therapy with 512 patients without treatment in a single clinical center, who were adjusted for background features by the multivariate analysis. Therefore, the principal aims of our study were to show the role of IFN in preventing HCC in chronic hepatitis type C en masse and to establish the extent to which IFN decreases the rate of carcinogenesis as a sequel to chronic hepatitis C in a society.

Patients and Methods

Study Population

A total of 2,166 patients with chronic hepatitis were examined, whose initial sera tested negative for hepatitis B surface antigen by radioimmunoassay (Ausria, Dainabot, Tokyo, Japan) and positive for anti-HCV by the second-generation enzyme-linked immunosorbent assay (Dainabot); anti-HCV was tested in sera that had been stored frozen at −80°C. They included 1,421 men and 745 women aged 14–78 with a median of 50 years. They were all diagnosed with chronic hepatitis by liver biopsy with or without peritoneoscopy between 1970 and 2000 at the Department of Gastroenterology in Toranomon Hospital, Tokyo, Japan. Patients who had possibly developed HCC already at the time of diagnosis of hepatitis were strictly excluded from the study. In order to exclusively investigate hepatocarcinogenesis in HCV-related cirrhosis, patients coinfected with HBV were excluded.

Among the 2,166 patients with HCV-related hepatitis, 1,654 (76.4%) received IFN therapy, mostly since 1987 when IFN was available in Japan; new antivirals or anticarcinogenic treatments of viral cirrhosis, except for IFN, were not introduced in 1987 or thereafter in Japan. The remaining 512 patients did not receive IFN or any other antiviral therapies. This is a retrospective cohort study with historical controls composed of patients before 1987 and those who refused or could not receive IFN for various reasons since 1987.

Background and Laboratory Findings

Table 1 shows demographic profiles and laboratory data for the 1,654 patients treated with IFN and the 512 without receiving IFN since they were diagnosed with chronic hepatitis. There were more males, with a median age 3 years lower in treated than in nontreated patients. There were 299 treated patients (18.1%) with a history of alcohol intake ≥ 500 kg until the diagnosis of chronic hepatitis (corresponding to daily consumption of 3,000 ml of beer or 300 ml of whiskey for 20 years) and 113 (22.1%) untreated patients ($p < 0.001$). Because IFN was introduced to our hospital in 1987, the observation period was significantly shorter in the treated than in untreated patients (median 10.4 vs. 12.3 years; $p < 0.0001$).

Although all patients tested positive for HCV RNA during their clinical courses, tests for the concentration of HCV RNA in the initial serum was possible in 1,863 (86.5%) patients. HCV genotypes were analyzed by the serological typing method with a commercial kit (Kokusai Diagnostic Corporation, Kobe, Japan) in which the serological group 1 represented genotypes 1a and 1b, and group 2 stood for 2a and 2b genotypes. HCV in the serological group 2 was significantly more frequent in patients with IFN treatment than in those without. Concentration of HCV RNA was determined in the initial sera from 1,873 (86.5%) patients by the competitive polymerase chain reaction (PCR) method with the HCV probe assay (Chiron Corp., Emeryville, Calif., USA) or by PCR with Amplicor HCV Monitor kits (Roche Diagnostics Japan Co., Tokyo, Japan). High concentration of HCV ($\geq 10^6$ copies/ml by the competitive PCR or $\geq 10^6$ equivalents/ml by the HCV probe assay) was significantly more frequent in untreated than in treated patients ($p < 0.0001$). The stage of hepatic fibrosis was not different between the two groups.

Interferon Treatment and Judgment of the Effect

A total of 1,654 patients underwent IFN therapy in one or more treatment courses: 1,358 patients (82.1%) received IFN once, 240 patients (14.5%) twice, and the remaining 56 patients (3.4%) three times or more. Initial treatment was performed with natural or recombinant IFN-α (n = 1,238), natural IFN-β (n = 386) or both (n = 30). Regimens of IFN were variable: 926 (56.0%) patients received IFN 6–9 million units (MU) daily for 8 weeks, followed by 2 or 3 times per week for 16 weeks; 329 (20.0%) received IFN 6–9 MU daily for 2–4 weeks, followed by 3 times per week for 20–22 weeks; 185 (11.2%) underwent a short-course therapy with IFN

Table 1. Patient profiles and laboratory data at the diagnosis of chronic hepatitis

Factors	Interferon therapy		p value
	yes (n = 1,654)	no (n = 512)	
Male	1,110 (67.1%)	311 (60.7%)	0.024
Age, years	50 (16–72)	53 (21–78)	<0.001
History of transfusion	607 (36.7%)	229 (44.7%)	0.001
Family member with liver disease	426 (25.8%)	140 (27.3%)	0.47
Alcohol intake ≥ 500 kg	299 (18.1%)	113 (22.1%)	0.044
Observation period, year	10.4 (0.1–33.6)	12.3 (0.1–33.6)	<0.001
Laboratory data			
ALT, IU/l	63 (4–1,266)	67 (4–704)	0.098
AST, IU/l	106 (9–1,660)	96 (12–832)	0.0001
γ-GTP, IU/ml	62 (6–1,118)	70 (3–850)	0.39
Platelet counts, × 1,000/mm^3	169 (27–433)	165 (35–560)	0.091
ICG R_{15}, %	14 (1–90)	16 (1–95)	0.003
AFP, ng/ml	4 (1–90)	5 (1–1,180)	0.42
HCV serological group			
Group 1, genotypes 1a/1b	1,021 (66.1%)	259 (81.4%)	<0.0001
Group 2, genotypes 2a/2b	488 (31.6%)	48 (15.1%)	
Undetermined	36 (2.3%)	11 (3.5%)	
HCV RNA concentration			
High[a]	937 (58.4%)	191 (71.3%)	<0.0001
Low[b]	668 (41.6%)	77 (28.7%)	
Histological stage of hepatitis			
F1, slight fibrosis	1,029 (62.2%)	298 (58.2%)	0.10
F2/F3, moderate/severe fibrosis	625 (37.8%)	214 (41.6%)	

AST = Aspartate aminotransferase; AFP = α-fetoprotein; ICG R_{15} = retention of indocyanine green at 15 min.

[a] HCV RNA concentration ≥ 10^6 copies/ml by the competitive PCR or ≥ 10^6 equivalents/ml by the HCV probe assay.

[b] HCV RNA concentrations less than high concentrations.

daily for 4–8 weeks; 128 (7.7%) were administered with intermittent IFN 3 times per week for 24 weeks; 72 (4.4%) had a prolonged course of IFN for 8–36 months; 8 (0.5%) received IFN-β 6 MU daily for 6–18 months, and the remaining 6 (0.4%) were given IFN-α combined with IFN-β for 4 months. The median dose of 624 MU was administered during the median period of 24 weeks. IFN for 24 weeks or longer was given to 83.2% of the patients. IFN therapy was usually initiated within a few months after the diagnosis of chronic hepatitis, and all patients were started on it within 12 months. The median interval between liver biopsy and initiation of IFN was 9 days.

Almost all the patients given IFN showed varied degrees of fever, chills, myalgia, headache and general malaise after the first injection. Most patients developed leukocytopenia and thrombocytopenia in various degrees. A significant thrombocytopenia ≤ 40,000/mm^3 required a reduction of the IFN dose in 39 patients. IFN therapy was discontinued due to psychosis in 35 patients and ophthalmological symptoms in 12 patients. None of the patients developed decompensated liver disease with ascites, encephalopathy, jaundice or variceal bleeding. Although only 88 (5.3%) patients could not continue injection with IFN, studies for carcinogenesis were analyzed on the intention-to-treat basis.

The efficacy of IFN was judged by the clearance of HCV RNA from serum and ALT levels 12 months after the completion of treatment. Sustained virological response (SVR) was defined as persistent disappearance of HCV RNA after therapy, biochemical response (BR) as normal ALT levels without elimination of HCV RNA for at least 6 months after therapy, and no response (NR) as persistently elevated or transiently normalized ALT levels without loss of HCV RNA lasting for less than 6 months.

Follow-Up of Patients and Diagnosis of HCC

Patients were followed up monthly after diagnosis of chronic hepatitis in our outpatient clinic and monitored for hematological, biochemical and virological parameters. With their admission, during and after the treatment with IFN, weekly or biweekly follow-up was performed in almost all patients who received IFN. Imaging diagnosis was made once or twice per year in the majority of patients with ultrasonography or computed tomography. Angiography was performed only when HCC was highly suspected on imaging by ultrasonography or computed tomography.

When angiography pictured a characteristic hypervascular nodule specific for HCC in patients, histological confirmation was not required in the majority of them. Microscopic examinations of liv-

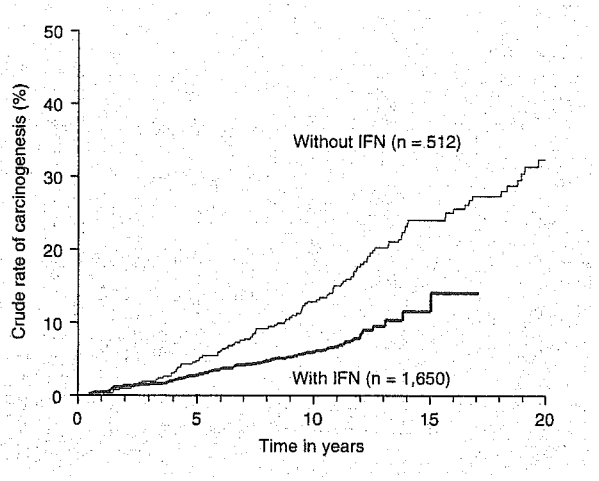

Fig. 1. Crude rates of hepatocarcinogenesis in patients treated with IFN and those untreated. The carcinogenesis rate was significantly lower in treated than in untreated patients (log-rank test, $p < 0.0001$).

er tissues obtained by a fine-needle biopsy were performed in 14 patients whose angiogram could not portray a typical image of HCC. There were 89 patients in whom HCC was confirmed histologically on liver specimens obtained at surgery or autopsy. Detection of serological tumor markers and increase with time were also taken into account in the diagnosis of HCC.

There were 223 (10.3%) patients lost to follow-up, including 164 (9.9%) treated and 59 (11.5%) untreated. Rates of annual dropouts in treated and untreated patients were 0.95 and 0.93%, respectively. In 9 patients, the response to IFN was judged by information on aminotransferase levels determined in other clinics and by persistent HCV RNA, as well as aminotransferase levels at 6 months after the completion of therapy in an additional 3 patients. Therefore, the response to IFN could be judged in all patients including the 12 who were lost to our follow-up early. Since the eventual outcome with respect to the development of HCC was not confirmed in these patients, their data were censored in statistical analyses [21]. Deaths unrelated to liver disease were censored and withdrawn from the analysis. The date of the last follow-up in this study was May 1, 2004, and the median observation period of studied patients was 10.7 years, with a range of 0.1–33.6 years.

Statistical Analysis

Nonparametric Mann-Whitney U test and χ^2 test were used for analysis of background characteristics of patients. The rate of HCC development was calculated by the Kaplan-Meier method [22]; it was based on the duration between diagnosis of chronic hepatitis by liver biopsy and detection of HCC. Differences in slopes of carcinogenesis curves were evaluated by the log-rank test. To gain a robust statistical power for the anticarcinogenic activity of IFN, observation of treated patients was initiated at the commencement of IFN therapy, in lieu of the diagnosis of chronic hepatitis. Independent factors associated with the development of HCC were studied using the stepwise Cox regression analysis [23]. The following 18 variables were analyzed for potential covariates in hepatocarcinogenesis at the time when hepatitis was diagnosed: age, sex, total alcohol intake, family history of liver disease, history of blood transfusion, stage of hepatic fibrosis, aspartic aminotransferase, ALT, albumin, bilirubin, globulin, γ-glutamyl transpeptidase (γ-GTP), platelet count, retention of indocyanine green at 15 min, serological grouping of HCV, HCV RNA level and IFN treatment.

Although continuous variables without conversion of data were evaluated in multivariate analyses, several variables were transformed into categorical data consisting of two or three ordinal numbers in calculating hazard ratios. All factors found to be marginally associated with hepatocarcinogenesis with p values <0.15 were tested by the multivariate Cox proportional hazard model. All analyses of data were performed with the computer program SPSS version 11 [24], and a p value <0.05 was considered significant.

Results

Response to IFN

Response to IFN was judged 12 months after the completion of therapy by both HCV RNA and serial ALT readings. Among the 1,654 patients with IFN treatment, SVR (elimination of HCV RNA) was achieved by 606 (36.6%), BR (ALT normalized for at least 6 months without clearance of HCV RNA from serum) in 266 (16.1%) and NR (elevated or transiently decreased ALT levels without loss of serum HCV RNA) in 782 (47.3%).

Crude Rates of Hepatocarcinogenesis

During the median observation period of 10.7 years, HCC developed in 199 of the 2,166 (9.2%) patients, including 96 of the 1,654 (5.8%) patients treated with IFN and 103 of the 512 (20.1%) patients without IFN (fig. 1). Among the 199 patients with HCC, 140 (70.4%) imaged a typical hypervascular stain on angiography and dynamic computed tomography, while 59 failed to exhibit tumor stains on angiography. HCC in these 59 patients was confirmed histologically on liver specimens obtained at surgery or by fine-needle biopsy.

Crude rates of hepatocarcinogenesis in patients treated with IFN and those untreated were 1.3 and 1.8% at the end of the 3rd year (after the completion of therapy), 2.6 and 4.6% at the end of the 5th year, 5.8 and 12.7% at the 10th year and 13.9 and 23.9% at the 15th year, respectively (fig. 1). The carcinogenesis rate was significantly lower in patients treated with IFN than in untreated patients (log-rank test, $p < 0.0001$).

Impact of IFN on Hepatocarcinogenesis

During the observation period, HCC developed in 96 of the 1,654 (5.8%) patients treated with IFN, including

11 patients (1.8%) with SVR, 10 (3.8%) with BR and 75 (9.6%) with NR to IFN. Rates of hepatocarcinogenesis in patients with SVR, BR and NR were 0.7, 0.8 and 2.0% at the end of the 3rd year, 1.4, 2.0 and 3.8% at the 5th year, 1.6, 2.9 and 6.5% at the 7th year, 1.9, 3.6 and 9.6% at the 10th year and 1.9, 7.5 and 27.6% at the end of 15th year (fig. 2). Hepatocarcinogenesis was significantly less frequent in patients with SVR or BR than in patients with NR and those untreated (log-rank test, $p < 0.0001$).

Factors Influencing Hepatocarcinogenesis

Univariate analysis identified 9 factors significantly associated with carcinogenesis. They were fibrotic stage ($p < 0.001$), age ($p < 0.001$), α-fetoprotein ($p < 0.001$), aspartic aminotransferase ($p = 0.001$), retention of indocyanine green at 15 min ($p = 0.002$), total alcohol intake ($p = 0.002$), γ-GTP ($p = 0.005$) and HCV serotype ($p = 0.045$). IFN therapy ($p = 0.064$), histological activity of hepatitis ($p = 0.069$) and ALT ($p = 0.70$) were marginally associated with carcinogenesis.

In order to prove the role of IFN on carcinogenesis in patients with chronic hepatitis type C en masse, multivariate analysis was performed by non-time-dependent proportional hazard analysis. Fibrotic stage, γ-GTP, gender, IFN therapy, platelet count and age independently influenced the development of HCC in the cohort (table 2). Advanced liver fibrosis in F2/F3 stages imposed a higher risk for carcinogenesis with a hazard ratio of 8.68, 95% confidence interval (CI) 5.08–14.81, compared with the F1 stage. Similarly, higher γ-GTP levels (hazard ratio 2.64), male sex (2.38), low platelet count (2.22) and older age (1.90) posed higher carcinogenesis risks. After adjusting background clinical biases between treated and untreated patients for the 5 significant covariates identified in the multivariate analysis, IFN therapy significantly decreased the hepatocarcinogenesis rate in the entire patients with chronic hepatitis C with a hazard ratio of 0.42 (95% CI 0.29–0.61) in comparison with untreated patients.

Based on the multivariate analysis, curves of carcinogenesis rates were theoretically illustrated in treated and untreated patients with the average histological stage, average γ-GTP value, average ratio of male to female, average platelet count and average age (fig. 3).

Hazard of Hepatocarcinogenesis Stratified by the Response to IFN

Since the carcinogenesis rate in patients with SVR or BR was significantly lower than that of patients with NR or untreated patients by the product limit method, a mul-

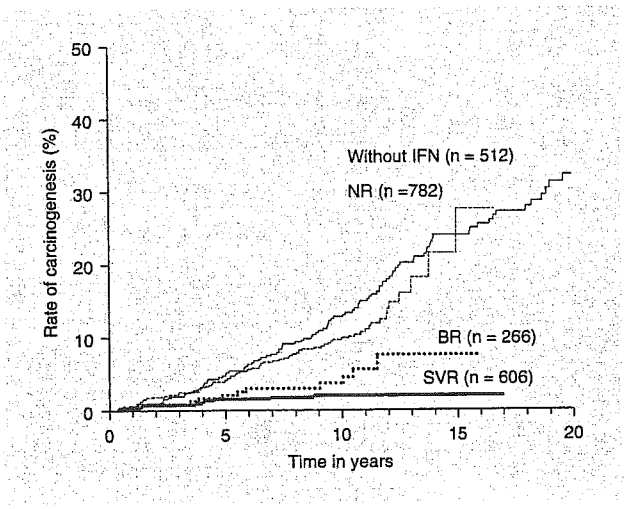

Fig. 2. Rates of hepatocarcinogenesis in patients with SVR, BR and NR to IFN. The rate in patients with NR (persistently elevated ALT or transiently normalized ALT for less than 6 months) was significantly higher than that in patients with SVR or BR.

Table 2. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C[a]

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	8.68	(5.08–14.81)	<0.001
γ-GTP, IU/ml			
<50	1		
≥50	2.64	(1.58–4.42)	<0.001
Gender			
Women	1		
Men	2.38	(1.56–3.70)	<0.001
IFN therapy			
No	1		
Yes	0.42	(0.29–0.61)	<0.001
Platelet count, $\times 10^3/mm^3$			
≥100	1		
<100	2.22	(1.47–3.44)	<0.001
Age, years			
<50	1		
≥50	1.90	(1.27–2.85)	0.002

HR = Hazard ratio.
[a] Evaluated by the Cox proportional hazard analysis.

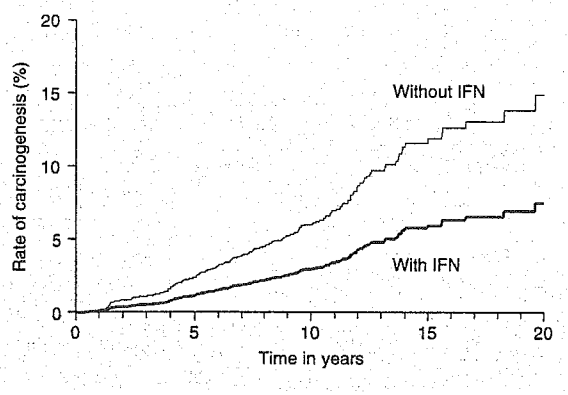

Fig. 3. Theoretical curves of hepatocarcinogenesis in patients treated with IFN and those untreated who have the average histological stage, average γ-GTP value, average ratio of male to female, average platelet count and average age. They are based on the analysis of 1,654 patients treated with IFN and 512 untreated patients.

Table 3. Factors associated with hepatocarcinogenesis in patients with chronic hepatitis C who had distinct responses to IFN therapy[a]

Factors	HR	95% CI	p value
Fibrosis stage			
F1	1		
F2–F3	9.90	(4.19–23.40)	<0.001
Gender			
Women	1		
Men	3.44	(1.89–6.25)	<0.001
γ-GTP, IU/ml			
<50	1		
≥50	2.68	(1.30–5.54)	0.008
Age, years			
<50	1		
≥50	2.56	(1.50–4.38)	0.001
AFP, ng/ml			
<20	1		
≥20	2.32	(1.34–4.02)	0.003
Platelet count, $\times 10^3/mm^3$			
≥100	1		
<100	2.09	(1.14–3.75)	0.013
Response to IFN			
Without IFN	1		<0.001
NR	0.57	(0.13–2.56)	0.46
BR	0.12	(0.04–0.35)	<0.001
SVR	0.10	(0.03–0.30)	<0.001

HR = Hazard ratio; AFP = α-fetoprotein.
[a] Evaluated by the Cox proportional hazard analysis.

tivariate analysis was performed taking into account the response to IFN. Hazard ratios of patients with SVR and BR to IFN therapy were 0.10 (95% CI 0.03–0.30, $p < 0.001$) and 0.12 (95% CI 0.04–0.35, $p < 0.001$), respectively, in comparison with that of untreated patients, when the other 5 factors served as significant covariates (table 3). The hazard ratio of NR at 0.57 (95% CI 0.13–2.56) was less than 1, but fell short of making a significant difference against untreated patients.

Mortality and Causes of Death

During the observation period, 116 of the 2,166 (5.4%) patients died, including 52 of the 1,654 (3.1%) subjects treated with IFN and 64 of the 512 (12.5%) subjects without IFN. Estimated survival rates in the treated and untreated patients were 99.3 and 98.3% at 5 years, 97.8 and 96.0% at 10 years and 93.8 and 86.9% at 15 years, respectively. The survival rate of treated patients was significantly higher than that of untreated patients (log-rank test, $p < 0.0001$).

Discussion

Based on our epidemiological data obtained by long-term observations of patients with chronic hepatitis [2] and patients with cirrhosis [1], the life expectancy of patients with HCV-related chronic liver disease heavily depends on the development of HCC. The possibility of eventually developing HCC in patients with HCV infection and cirrhosis is staggeringly high at 75% [1]. Theoretically, the treatment of chronic HCV infection with IFN can prevent the development of HCC. From the ethical point of view, a prospective randomized trial with control untreated patients is not to be allowed at present when IFN has become the standard radical therapy for chronic hepatitis C; everyone can receive IFN, as expenses are being covered for by the medical insurance in Japan. Another difficulty involves the informed consent in prospective randomized studies. It requires at least 5 years in order that IFN can decrease the incidence of carcinogenesis in chronic hepatitis C, with a statistical difference in the carcinogenesis rate between treated and 'untreated' patients. Since any randomized studies are considered extremely difficult in the future, we attempted to carry out this retrospective study by the multivariate analysis with statistical adjustments for possible covariates.

In the product limit analysis, IFN significantly decreased the crude rate of hepatocarcinogenesis in the

entire cohort of 2,166 patients with chronic hepatitis C. Since there were some background differences between treated and untreated patients, we tried to correct for biases including stage of fibrosis, γ-GTP value, sex, platelet count and age, which significantly affect the carcinogenesis rate. Demographic, histological and biochemical factors having been adjusted, IFN is proven to bring about a significant decrease in the hazard of carcinogenesis in patients with chronic hepatitis C en masse (hazard ratio 0.42, $p < 0.001$ by the non-time-dependent model). Taking into consideration that a significant number of patients without IFN had received anti-inflammatory medicines, which might have contributed to suppression of hepatocarcinogenesis, the actual anticarcinogenic activity of IFN may be higher than the observed. Having published results of a similar study on a cohort of 1,643 patients with a median observation period of 5.4 years in 1999 [18], we could not establish the anticarcinogenic activity of IFN because of a low risk of carcinogenesis in untreated patients (1.2% per year). Nevertheless, we expected a significant statistical difference if we could extend the median observation period to longer than 7 or 10 years in our studied patients. This has been realized in the present study, in which 2,166 patients with and without IFN therapy were observed for a median of more than 10 years. As far as we are aware, it represents the first study that has demonstrated preventive effects of IFN on the carcinogenesis rate in a large cohort of patients in a single center, in correlation with distinct responses to it, such as SVR, BR and NR.

Treatment of patients with chronic HCV infection using IFN-α and ribavirin has led to sustained loss of serum HCV RNA in 40–50% of recipients with HCV genotype 1 and 75–80% with HCV genotype 2 or 3. However, to date, the combination therapy with IFN-α and ribavirin has not been evaluated for its impact on the risk of developing HCC. Monotherapy with IFN-α achieves sustained clearance of serum HCV RNA in only 20–30% of patients; the impact of IFN-α on the development of HCC has been evaluated only in patients who had received IFN-α without ribavirin [17–20, 25–27].

Multivariate analysis definitively demonstrated that IFN lessens the carcinogenesis risk in the patients whose ALT levels decreased after therapy. Furthermore, the anticarcinogenic capacity of IFN was demonstrated not only in the patients with persistent aminotransferase normalization, but also in those with transient normalization of ALT for at least 6 or 12 months. Many authors have already described that the activity of IFN to suppress the development of HCC in patients with HCV RNA clearance (SVR) is similar to that in patients with ALT normalization in the absence of eliminating HCV RNA (BR) [18, 25–27]. Based on these compelling lines of evidence, the anticarcinogenic activity of IFN is ascribed to the suppression of inflammatory and regenerative processes in hepatocytes. Moreno and Muriel [28] reported that IFN reverts liver fibrosis, and therefore, control of the necroinflammatory process can suppress the growth of HCC. Tarao et al. [29] reported that high aminotransferase levels increase the rate of HCC recurrence in patients with cirrhosis. Our results stand in favor of the view that the carcinogenic process in patients with chronic hepatitis C would be enhanced by fluctuating as well as persistently elevated levels of aminotransferases. It does seem that IFN exerts suppressive effects on HCC through reduction or complete remission of inflammatory activity. Recently, a few authors reported that even transient disappearance of HCV RNA during IFN therapy contributed to a low carcinogenesis rate in the clinical course of hepatitis [17, 27]. The significance of transient HCV in decreasing hepatocarcinogenesis should be further explored and confirmed by multicenter clinical studies with rigorous virological assessments.

HCC developed in a few patients with SVR 5 years after the HCV infection had been terminated by IFN, along with normalized ALT levels. These patients would have developed minute HCC in their livers already while receiving IFN which escaped the detection by imaging modalities or screening for serological tumor markers. This would indicate the limitation of IFN in preventing HCC. IFN will not be able to suppress HCC once it has developed, even when it succeeds in eliminating HCV and suppressing necroinflammatory processes in the liver.

With many difficulties in vaccine development, the recent progress in treatment of chronic HCV infection, from IFN monotherapy to combination therapy with ribavirin, is very auspicious. SVR and BR can be achieved in up to 56% of patients with combined IFN and ribavirin [30]. There is evidence that a sustained virological response can lead to decrease in fibrosis and even reversal of cirrhosis [31]. Because HCV-associated HCC occurs almost exclusively in patients with cirrhosis, successful treatment for SVR in patients without cirrhosis is likely to prevent future development of HCC [32]. However, once cirrhosis has been established, a preventive benefit of IFN monotherapy is restricted to the patients who can achieve SVR or BR. In their meta-analysis of 3 randomized and 11 nonrandomized controlled trials, Camma et

al. [33] have reported a low but statistically significant preventive effect.

In conclusion, IFN significantly decreases the rate of hepatocarcinogenesis in patients with chronic hepatitis C, irrespective of the response to it.

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