Development of hepatocellular carcinoma in patients with chronic hepatitis C more than 10 years after sustained virological response to interferon therapy

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Abstract. The risk factors for the development of hepatocellular carcinoma (HCC) in patients who have achieved a long-term sustained viral response (SVR) to interferon (IFN) are not fully understood. This study aimed to investigate the characteristics of patients who developed HCC after 10 years of achieving SVR. We retrospectively studied 5 patients with HCC which developed more than 10 years after the termination of IFN therapy. The clinical characteristics at the induction of IFN therapy were male gender, a mean age of 51.6±9.1 years, while 2 patients were moderate alcohol consumers. None of the 5 patients were positive for either HBs Ag or anti-HBc Ab. A histological examination at the initial IFN therapy showed the activity scores to be A2 in all cases, and the fibrosis scores at least F2. The clinical parameters at the diagnosis of HCC included fluctuating transaminase levels in all cases. These levels scarcely fell below the upper limits even after SVR was achieved. In 3 patients, liver tissues were obtained at the treatment of HCC. These tissues showed marked improvement in both activities and fibroses, but severe steatosis in 1 patient. To conclude, chronic hepatitis C patients who respond to IFN therapy should undergo long-term follow-up, even after the eradication of HCV, with special attention particularly to patients who had elevated transaminase levels and steatosis.

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Introduction

Hepatocellular carcinoma (HCC) is the major cause of cancer-related death in Japan. Approximately 70-80% of HCCs in Japanese patients are associated with hepatitis C virus (HCV) infection (1). HCV causes chronic infection in more than 70% of cases, and liver disease gradually progresses to liver cirrhosis and finally to HCC.

Interferon (IFN) has been used for the treatment of chronic hepatitis C (CHC) patients. Many investigators have reported that IFN treatment is effective in the reduction of the serum alanine amino transferase (ALT) level, eliminating HCV RNA from the circulation and improving liver histology in CHC patients (2-6). In certain patients, IFN therapy normalizes the serum ALT levels and leads to sustained eradication of HCV. These patients are commonly referred to as having achieved a sustained viral response (SVR) (7), and it has been noted that the cumulative incidence of HCC is significantly lower in SVR patients than in those with a non-response (NR) to IFN therapy (8,9), suggesting that the success of treatment for HCV infection is expected to significantly reduce the risk of developing HCC.

However, the development of HCC among CHC patients with SVR to IFN therapy has been reported (10-16). In most cases, HCCs occurred within 5 years after the termination of IFN treatment. The risk factors for developing HCC after achieving SVR were suggested in these reports; however, the associated significant factors remain unknown. Moreover, the risk factors for the development of HCC in patients who have achieved SVR for more than 10 years are not fully understood. Therefore, it remains undetermined which patient groups should undergo long-term follow-up after SVR to IFN therapy for the risk of HCC. Recently, we identified 5 patients who developed HCC more than 10 years after SVR to IFN therapy. In this study, we investigated the characteristics of these patients.

Table I. Clinical characteristics of 5 patients at the INF induction.

Case no.	Gender	Age	Blood transfusion	Alcohol intake (g/day)	HBs-Ag	HBs-Ab	AST (IU/l)	ALT (IU/l)	PLT (10^4/mm^3)	T-Cho (mg/dl)	AFP (ng/ml)	Histology
1	Male	62	(+)	(-)	(-)	(-)	90	123	21.3	162	6.6	A2/F2
2	Male	59	(-)	43.2	(-)	(-)	244	153	10.8	169	22.5	A2/F2
3	Male	44	(+)	43.2	(-)	(-)	56	97	13.2	N/A	N/A	A2/F3
4	Male	52	(+)	(-)	(-)	(-)	194	172	15.5	179	N/A	A2/F3
5	Male	41	(+)	N/A	(-)	(-)	106	114	9.6	164	21.8	A2/F3

AST, ; ALT, alanine amino transferase; PLT, ; T-Cho, ; AFP α-fetoprotein.

Table II. Clinical characteristics of 5 patients at the HCC onset.

Case no.	Years after IFN	AST (IU/l)	ALT (IU/l)	PLT (10^4/mm^3)	AFP (ng/ml)	Histology	Tumor size (mm)	No.
1	18	35	27	21.5	4.4	A0/F2	25	2
2	18	83	66	9.5	2.0	A0/F1	21	1
3	11	35	40	10.9	4.9	N/A	25	1
4	15	40	21	17.8	1,931.1	N/A	N/A	Multiple

IFN, interferon; AST, ; ALT, alanine amino transferase; PLT, ; AFP α-fetoprotein.

Patients and methods

Patients. Between 1992 and 2000, a total of 674 patients with chronic HCV infection were treated with IFN (6-10 million units of IFN-α or -β daily for 2-4 weeks, followed by 6-10 million units of IFN three times a week for 20-22 weeks). We were able to follow 464 of the 674 patients; 142 of these 469 patients attained continuous normalization of the serum ALT level. Moreover, the disappearance of HCV RNA from the serum was determined by a nested reverse transcription-polymerase chain reaction (RT-PCR) assay or the Amplicor HCV monitor assay (Roche Molecular System, Pleasanton, CA, USA) at 6 months after termination of IFN therapy. HCV was considered to be eradicated in these patients, and they achieved a sustained viral response (SVR). Ninety-two patients presented with a positive serum HCV RNA, but with a normal serum ALT level at the end of treatment. They were defined as achieving a biological response (BR). In 230 patients, the serum HCV RNA was positive while the serum ALT level was elevated after termination of the IFN therapy. These patients demonstrated non-response (NR).

Follow-up and diagnosis of HCC. Follow-up of the patients consisted of blood examinations including ALT, AST, PLT and α-fetoprotein (AFP) at regular intervals of 1-3 months. To detect HCC, diagnostic imaging was performed every 6 months by ultrasonography (US). Computed tomography (CT) was performed once a year. The diagnosis of HCC was made using liver imaging (US, CT or magnetic resonance imaging) and/or angiography. In patients whose angiogram did not demonstrate a typical hypervascular image of HCC, a microscopic examination of liver specimens obtained by echo-guided needle biopsy was performed. Liver biopsy was performed before IFN induction and HCC onset. Histological diagnosis was carried out according to the Metavir scoring system.

Results

A total of 464 patients underwent IFN therapy between 1992 and 2000. Of these, 142 (30.7%) achieved SVR, 92 (19.9%) ended the therapy with BR and 230 patients (49.8%) demonstrated NR. Eleven patients with SVR developed HCC during follow-up. In 5 patients, HCC was detected more than 10 years after the end of the IFN therapy.

The clinical characteristics of the 5 patients at the induction of IFN therapy are listed in Table I. All patients were male and their mean age was 51.6±9.1 years. Two patients were moderate alcohol drinkers with an intake of 43.2 g ethanol per day. Four patients had a history of previous blood transfusion. None of the 5 patients were positive for either HBs Ag or anti-HBc Ab. A histological examination showed the activity scores to be A2 in all cases, and the fibrosis scores at least F2.

Table II shows the clinical parameters of the 5 patients at the diagnosis of HCC. The mean interval from the end of therapy to the detection of HCC was 15.4±2.9 years. The transaminase levels fluctuated in all 5 cases, and scarcely fell below the upper limits even after SVR was achieved. The PLT levels improved in 3 patients. In 3 patients, liver tissues were obtained during treatment of HCC. A histological examination of these 3 patients showed marked improvement in both

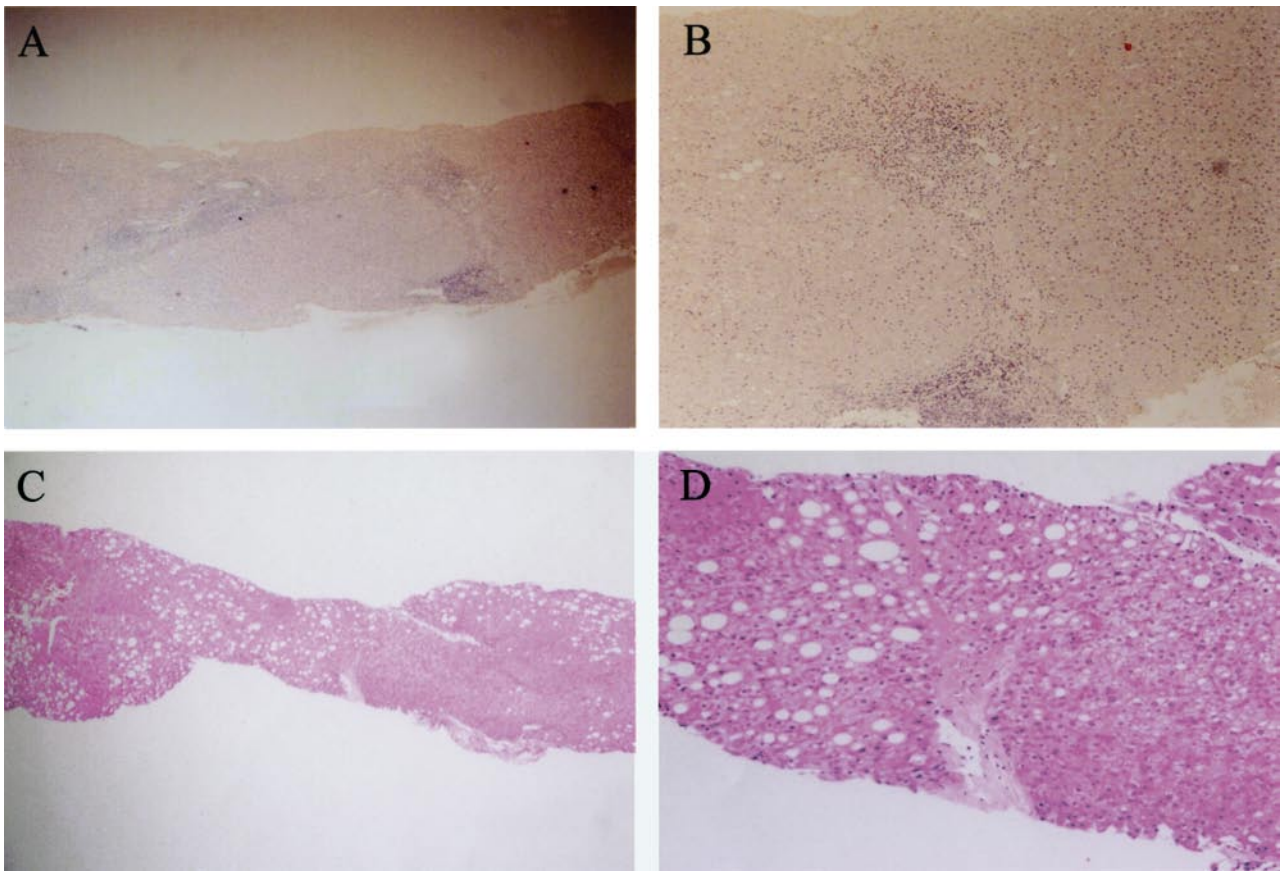

Figure 1. (A and B) Histological findings at the induction of IFN presenting chronic hepatitis A2/F2 (A, magnification, x40; B, x100). (C and D) Histological findings upon detection of HCC, presenting chronic hepatitis A0/F1; severe drop-related fatty degeneration is outstanding (C, magnification, x40; D, x100).

activities and fibroses. The histological findings in case no. 2 are shown in Fig. 1. In this case, the diagnosis of A2/F2 was made histologically before IFN therapy (Fig. 1A and B), and the scores significantly decreased to A0/F1 upon detection of HCC 18 years after IFN therapy (Fig. 1C and D). Moreover, notable macrovesicular fat depositions were observed in the hepatocytes of the second biopsy specimen.

Four patients underwent successful transcatheter arterial chemoembolization (TACE) and radiofrequency ablation for the therapy of HCCs. Only case no. 5 succumbed to the disease due to the progression of HCC and metastasis to the lung and bones.

Discussion

The cumulative incidences of HCC in IFN-treated patients are significantly low compared to non-treated patients, particularly in the F3 and F4 groups (5). This suggests that IFN reduces the risk of HCC even in the non-SVR patient group. In the present study, 5 cases developed HCC more than 10 years after the eradication of HCV by IFN therapy. The clinicopathological findings of these patients included male gender and age at treatment of over 40 years. In addition, the histological examination showed the fibrosis score of each case to be at least F2, and the serum ALT levels were elevated in all cases even after SVRs were achieved. Previous studies suggest that older age, male gender and advanced hepatic fibrosis are linked to an increased risk for the development of HCC among patients with SVR. These factors are consistent with our findings. We did not determine the accurate incidence of HCC in the SVR group as many patients were unable to be followed up after the end of the treatment. One retrospective study reported that 3.5% (13) of 373 SVR cases developed HCC. The mean interval from IFN therapy to the detection of HCC in this study was 5.8 years, which did not differ significantly from that in the non-SVR patient group (17).

One of the most important findings of the present study is that all 5 cases presented elevated ALT levels after SVR to IFN therapy, which was already suggested as a risk factor for HCC by a previous study. Although the reasons why ALT levels did not decrease below the normal range in these cases were not fully defined, we can speculate several possibilities. First, HCV may remain in the hepatocytes at a very low level causing persistent hepatitis. Maylin *et al* revealed that HCV RNA was detectable in 2 (1.7%) out of 114 liver specimens after SVR, though serum HCV RNA remained undetectable in all cases (18). However, HCV RNA is not integrated in host genome DNA and is not a carcinogen by itself without inflammation or fibrosis. Therefore, the scenario that HCV RNA remained in the hepatocytes to sustain hepatitis which ultimately caused HCC appears to be unlikely in our cases, since the histological examination showed that both activity and fibrosis significantly improved in the 3 cases in which liver biopsies at therapy for HCC were performed. A second

possibility for the increase in ALT levels is steatosis due to alcohol, diabetes mellitus (DM) or obesity. In the present study, 2 patients had a history of alcohol intake, 3 patients had DM and, the histological findings revealed that 2 patients had moderate steatosis in the liver cells. Therefore, we cannot eliminate the possibility that steatosis affected the occurrence of HCC to a certain degree in these cases.

In addition, HCC is associated with occult HBV infection. Although the anti-HBc antibody was negative in all of our cases, Tamori *et al* reported the integration of HBV DNA into the host genome in 4 out of 7 patients, 2 of whom tested negative for both anti-HBs Ab and anti-HBc Ab (20). Since we did not evaluate the HBV DNA in HCC cells or integration, whether HBV was related to the carcinogenesis in our cases needs to be ascertained. Further investigation is thus required to elucidate this issue.

Furthermore, the transformation of normal hepatocytes to cancer cells may occur before IFN therapy when the activities of inflammation are high. This may have occurred in the cases in which HCC developed within 5 years after SVR. Since HCC generally progresses very slowly, particularly at an early stage, and all of our cases were histologically well-differentiated HCCs (data not shown), it is possible that IFN had an effect on the differentiation of HCC cells and inhibited cell growth. This resulted in the HCCs being undetectable by diagnostic imaging for more than 10 years after carcinogenesis.

Although we did not define the accurate mechanism of the occurrence of HCC after a long period of SVR, we conclude that male gender, advanced fibrosis, older age at treatment and sustained elevation of ALT are potential risk factors for HCC. Moreover, in our cases, HCC may have developed even 18 years after SVR had been achieved. Therefore, CHC patients who respond to IFN monotherapy or combination therapy should undergo long-term follow-up, even after the eradication of HCV, with particular attention to patients who exhibit the above-mentioned risk factors, in order to detect small and controllable HCCs.

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