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Incidence of Hepatocellular Carcinoma after Interferon Therapy in Patients with Chronic Hepatitis C

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Onodera, H., Ukai, K., Suzuki, M. and Minami, Y. *Incidence of Hepatocellular Carcinoma after Interferon Therapy in Patients with Chronic Hepatitis C.* Tohoku J. Exp. Med., 1997, **181** (2), 275-283 —— We examined the effect of interferon (IFN) therapy for chronic active hepatitis (CAH) C in 207 patients by estimating the incidence of hepatocellular carcinoma (HCC) after IFN therapy using the person-years method. Statistical analysis was performed using the Mantel-Haenszel chi-square test. No HCC was detected in patients with normal serum alanine aminotransferase (ALT) levels after IFN therapy (response-effect group), and in patients with both normal serum ALT levels and hepatitis C virus (HCV)-RNA clearance after IFN therapy (complete-responder group). The incidence per 100,000 person-years in the patients with elevated serum ALT level after IFN therapy (other-effects group) and in the patients with positive HCV-RNA after IFN therapy (non-responder group) were 1968 and 1624, respectively. The incidence in control patients who did not achieve IFN therapy was 901. No statistically significant differences were observed between the other-effects group, non-responder group, and the control group. Our results so far suggest that normalization of the serum ALT levels and/or HCV clearance might reduce the incidence of HCC. —— interferon; chronic hepatitis C; hepatocellular carcinoma

Patients of chronic hepatitis (CH) C sometimes develop liver cirrhosis, which is considered a risk factor for hepatocellular carcinoma (HCC) (Realdi et al. 1982; Mattsson et al. 1988; Kiyosawa et al. 1990; Bisceglie et al. 1991). Recent investigators have reported that clearance of the hepatitis C virus (HCV) was obtained in about 40% of the CH C cases after interferon (IFN) therapy (Douglas et al. 1993; Shibata et al. 1993; Arase et al. 1994; Chayama et al. 1994; Lampertico et al. 1994), and that associated histological changes improved (Pérez et al. 1993; Arase et al. 1996; Tsuchihashi et al. 1996). According to the Liver Cancer Study Group of Japan, liver cirrhosis was observed in the noncancerous portion of the

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liver in 65.6% of Japanese HCC patients, and anti-HCV was positive in 68.9% of Japanese HCC patients (Liver Cancer Study Group of Japan 1995). We evaluated the incidence of HCC in a short-term observation study after the IFN therapy for CH C, and discussed the prevention of HCC using IFN therapy.

Patients and Methods

Since February, 1992, we have treated 235 patients with chronic active hepatitis (CAH) C using IFN therapy. In February, 1996 we evaluated the effectiveness of that therapy in an observational study that included 207 of those same patients. The ages of these 207 patients ranged from 23 to 69 years (51.6± 9.5 years, mean±S.D.). The male-to-female ratio was 1.34 (Table 1). In all cases, ultrasonically guided aspiration biopsy of the liver was performed 1 to 2 weeks before initiating IFN therapy. We employed 4-grade evaluation scale (Iino 1993). The four grades were response, partial response, aggravation and no-change. In response grade case, ALT levels returned to normal within 6 months after completion of therapy and remained normal thereafter for at least 6 months. In partial response grade case, ALT levels improved to less than two times the upper limit of normal within 6 months after completion of therapy and maintained such levels thereafter for at least 6 months. In aggravation grade case, ALT levels were clearly worse than pretreatment levels during the 6 months after completion of therapy. In no-change grade case, changes of ALT levels in response, partial response and aggravation grades were not applicable.

Although we evaluated the effect by four grades, we divided the patients into two groups; the response-effect group and the other-effects group. Other-effects included partial response, aggravation and no-change. Examination of serum HCV-RNA (nested reverse transcriptase polymerase chain reaction using primers derived from the 5′-untranslated region of HCV strain) was performed before and 6 months after IFN therapy. According to the results of HCV-RNA, we further divided the patients into two groups, the complete- and the non-responder groups. A complete responder was defined as a patient showing response grade and

Table 1. *Age and sex distributions of patients in our study who underwent IFN therapy*

Age (years)	Male	Female	Total
20–29	3	2	5
30–39	13	5	18
40–49	44	13	57
50–59	38	43	81
60–69	22	24	46
Total	120	87	207

Numbers of patients are indicated.

HCV-RNA negativity at 6 months after the termination of IFN therapy.

Natural IFN-α (Sumiferon, Sumitomo Pharmaceuticals Co. Ltd., Tokyo), recombinant IFN (rIFN)-α2a (Roferon, Nippon Roche K.K., Tokyo; Canferon, Takeda Chemical Industries Ltd., Osaka) or rIFN-α2b (Intron A, Schering-Plough K.K., Osaka; Intron A, Yamanouchi Pharmaceutical Co., Tokyo) was administered by intramuscular injection daily for 2 to 3 weeks, followed by three injections weekly for 10 to 24 weeks. Natural IFN-β (Feron, Daiichi Pharmaceutical Co. Ltd., Tokyo) was administered by intravenous injection daily for 6 weeks. We considered that total doses of 480 or more mega units (MU) of natural IFN-α, rIFN-α2a or rIFN-α2b and 252 or more MU of IFN-β, respectively, were sufficient. The total dose of 480 MU was composed of 6 MU IFN injected daily for 2 weeks, followed by three weekly injections of 6 MU IFN for 22 weeks. The total dose of 252 MU involved 6 MU IFN injected daily for 6 weeks. Sufficient total doses of natural IFN-α, rIFN-α2a or rIFN-α2b and IFN-$\beta$was administered in 73, 23, 15 and 35 patients, respectively. Insufficient total doses of natural IFN-α, rIFN-α2a or rIFN-α2b and IFN-β was administered in 28, 15, 16 and 2 patients, respectively (Table 2).

After termination of IFN therapy, 194 of the 207 patients were observed in our hospital outpatient clinic for chronic liver diseases. Thirteen patients dropped out during the observation. For the screening of HCC in the outpatient clinic, ultrasonography was performed at 3- to 4-month intervals and the levels of tumor markers (α-fetoprotein and PIVKA-II) were measured at 1- to 3-month intervals. When a patient was highly suspected of having HCC, CT, MRI, angiography and/or ultrasonically guided aspiration biopsy were performed to confirm the diagnosis.

For a control group, we included 140 patients with CH C, who were treated and observed in the outpatient clinic for chronic liver diseases and did not

TABLE 2. *The types of interferon (IFN), total doses, daily doses and the respective number of patients*

Interferon	Total dose (mega units)	Daily dose (mega units)	Numbers of patients
Natural IFN-α	480 or more	3 or 6	73
	Less than 480	3 or 6	28
rIFN[a]-α 2a	480 or more	3, 6 or 9	23
	Less than 480	3, 6 or 9	15
rIFN[a]-α 2b	480 or more	3, 6, 9 or 10	15
	Less than 480	3, 6, 9 or 10	16
Natural IFN-β	252 or more	3 or 6	35
	Less than 252	3 or 6	2

[a]recombinant IFN

undergo IFN therapy. The diagnosis of chronic hepatitis was obtained by serum liver function tests. The age of these patients ranged from 20 to 78 years (55.9± 12.7 years, mean±S.D.). The male-to-female ratio was 0.73 (Table 3).

We calculated the incidences of HCC after IFN therapy in the response group ($n=90$), the other-effects group ($n=117$), the complete-responder group ($n=59$), the non-responder group ($n=114$), and the control group. The incidences were estimated using the person-years method. Because IFN therapy for the first patient terminated on May 2, 1992, the observations began on this day, and the effects of IFN therapy was evaluated on February 29, 1996. The observation period in the control group was the same as that of the IFN treated patients.

Statistical analysis was performed using the Mantel-Haenszel chi-square test adjusted for sex and age. In the analysis the patients were divided into groups according to age as follows, 20–29, 30–39, 40–49, 50–59, 60–69 and 70–79 years. The statistically significant level was established at $p \leqq 0.05$.

Results

HCC was detected in 3 of the 140 control patients. The incidence per 100,000 person-years in the control group was 901. The 3 patients with HCC consisted of 2 males (ages 57, 61) and 1 female (age 71).

HCC was detected in 4 of the 207 patients who had undergone IFN therapy (Tables 4, 5). They were 3 males (ages 56, 66, 67) and 1 female (age 57). Liver histology prior to IFN therapy confirmed CAH 2b in all cases. The periods between the termination of IFN therapy and the discovery of HCC were 2, 3, 8, and 14 months. HCV-RNA genotype (Okamoto et al. 1992) was II in 2 patients and III in 2 patients. Three of these 4 patients were administrated insufficient total dose IFN. The fourth patient was administered a sufficient total dose of IFN, 252 MU of natural IFN-β. The incidences of HCC per 100,000 person-years in the patients who were administered sufficient and insufficient total dose IFN were 348 and 2864, respectively (Table 6). No statistically significant differences

Table 3. *Age and sex distributions of patients in the control group*

Age (years)	Male	Female	Total
20–29	1	5	6
30–39	7	7	14
40–49	8	7	15
50–59	22	22	44
60–69	12	30	42
70–79	9	10	19
Total	59	81	140

Numbers of patients are indicated.

TABLE 4. *Case summaries for hepatocellular carcinoma detected after interferon therapy for chronic hepatitis C*

Case No.	Age (years)	Sex	Periods[a] (months)	Numbers of tumors	Location of tumors	Size (cm)	Treatment	Survival time (months)
1	66	M	2	1	S_8	1.8	Resection	32 alive
2	56	M	3	2	S_8	1.0	Resection	29 alive
					S_8	1.0		
3	67	M	14	4	S_4	1.8	TAE[b] and PEI[c]	24 alive
					S_5	2.0		
					S_5	1.0		
					S_8	1.5		
4	57	F	8	1	S_7	0.9	PEI	19 alive

[a]Periods between the termination of interferon therapy and the discovery of hepatocellular carcinoma.
[b]Transcatheter hepatic arterial embolization.
[c]Ultrasonically guided percutaneous ethanol injection.

TABLE 5. *Case summaries for interferon (IFN) therapy in patients with hepatocellular carcinoma detected after IFN therapy for chronic hepatitis C*

Case No.	Age (years)	Sex	Histology[a]	HCV-RNA genotype[b]	Type of IFN	Total IFN dose (MU[c])	Effect of IFN
1	66	M	CAH2b	II	nIFN[d]-α	264	NR[g]
2	56	M	CAH2b	II	nIFN[d]-β	252	NR
3	67	M	CAH2b	III	nIFN[d]-α	36[f]	NR
4	57	F	CAH2b	III	rIFN[e]-α 2b	126	NR

[a]Histology at the beginning of interferon therapy.
[b]According to the typing established by Okamoto et al. (1992).
[c]Mega Units
[d]natural IFN
[e]recombinant IFN
[f]IFN therapy was discontinued due to the decrease of platelet count.
[g]non-responder

were observed between the patients who were administered sufficient total dose IFN, the patients who were administered insufficient total dose IFN, and the control patients.

No HCC was detected in the 90 patients of the response-effect group, and HCC was detected in 4 of the 117 patients of the other-effects group. The incidence per 100,000 person-years in the other-effects group was 1968 (Table 6). No statistically significant difference was observed between the other-effects group and the control group.

TABLE 6. *The incidences of hepatocellular carcinoma in our study patients who underwent interferon therapy and in the control patients*

Group	No. of patients	Person-years	Numbers of patients with HCC	Incidence of HCC[a]
Sufficient total dose IFN	146	287.50	1	348
Insufficient total dose IFN	61	104.50	3	2864
Response-effect	90	189.00	0	0
Other-effects	117	203.25	4	1968
Complete-responder	59	129.50	0	0
Non-responder	114	246.25	4	1624
Control	140	333.00	3	901

[a]Incidence of hepatocellular carcinoma (HCC) per 100,000 person-years

No statistically significant differences were obtained using the Mantel-Haenszel chi-square test. Statistically significance was established at $p \leqq 0.05$.

No HCC was detected in the 59 patients of the complete-responder group, and HCC was detected in 4 of the 114 patients of the non-responder group. The incidence per 100,000 person-years in the non-responder group was 1624 (Table 6). No statistically significant difference was observed between the non-responder group and the control group.

DISCUSSION

Recent advances in real-time ultrasonography have contributed to the early detection of HCC (Sheu et al. 1985; Liaw et al. 1986). HCC screenings using ultrasonography have been performed in our outpatient clinic for chronic liver diseases. Seventy-nine percent of the HCCs detected using this screening method were in stages I and II (Onodera et al. 1994). In our previous report (Onodera et al. 1995), we concluded that patients in the early stage of HCC associated with mild liver cirrhosis have a significantly better chance for long survival than the patients with HCC in stages III and IV. Of course prevention of the disease is the most important factor for reducing the mortality rate. Because HCV is recognized as having a close relationship with HCC in Japan (Kiyosawa et al. 1990; Saito et al. 1990; Yano et al. 1991; Takeda et al. 1992), the most important preventive measure is to stop HCV infection. It is also important to take preventive measures against HCC for the people who have been already infected with HCV. IFN is expected to reduce the incidence of HCC in the patients with chronic hepatitis C.

Nishiguchi et al. (1995) reported the incidence of HCC after IFN therapy. They concluded that IFN was associated with a decreased incidence of HCC. They administered IFN-α 3 times a week for 12 to 24 weeks, total dose was less than 432 MU. The incidence of HCC in IFN treated patients (4%) was significantly lower than in controls (38%). HCV-RNA did not disappear and serum ALT levels did not decrease after IFN therapy in any of the HCC patients.

In the present study, the incidence of HCC in the sufficient total dose IFN group was relatively but not significantly low as compared to the incidence in the insufficient total dose and the control groups. These results suggest that the administration of sufficient total dose IFN reduces the incidence of HCC. The incidence in the insufficient total dose group was 3 times that of the control group. Because we did not perform liver biopsy in the controls, some patients with chronic persistent hepatitis could have been included in the control group. This might account for the relatively low incidence of HCC in the control group.

Makita et al. (1996) reported 6 patients in whom HCC was detected and HCV-RNA remained positive after IFN therapy. In our study, HCV-RNA remained positive and serum ALT levels did not normalized in all HCC detected after IFN therapy. On the other hand, no HCC was detected in the response-effect group and the complete-responder group. Although the reason why IFN affects the incidence of HCC is not known, our results have so far implicated normalization of the serum ALT level and/or clearance of the HCV virus to be preventive against HCC. Although the results of this study were obtained by short-term observation, on the basis of our findings we recommended IFN therapy with sufficient total dose for CH C patients to prevent HCC.

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