EFFICACY OF INTERFERON THERAPY FOR CHRONIC HEPATITIS C — A COOPERATIVE STUDY IN ELEVEN HOSPITALS —

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Abstract: We investigated the influences of liver histology, serum levels of hepatitis C virus (HCV) and HCV genotypes on responsiveness to interferon (IFN) therapy in 342 patients with chronic hepatitis C. Either 9 million units (MU) of lymphoblastoid alpha IFN or 3 MU of recombinant IFN-alpha was administered daily for 2 weeks and then three times a week for 22 weeks. IFN responses were divided into three groups on the basis of the results of polymerase chain reaction (PCR) assay detecting HCV-RNA in serum. Complete response (CR) was defined as sustained elimination of HCV for at least 6 months after treatment, partial response (PR) as HCV elimination for a limited period, non-response (NR) as continuously positive for HCV-RNA in serum. Quantitation of pre-treament HCV-RNA amount in serum was determined by competitive PCR assay in 47 patients. HCV genotyping was performed in 114 patients by PCR with genotype-specific primers. CR was obtained in 97 patients (28.4 %), PR in 104 (30.4 %) and NR in 141 (41.2 %). IFN responses, represented by CR/PR/NR, were 15/18/11 in 44 patients with chronic persistent hepatitis (CPH), 72/65/73 in 210 patients with chronic aggressive hepatitis (CAH) 2 a, and 10/21/57 in 88 patients with CAH 2 b. CR rate was lower in patients with CAH 2 b (11.4 %) compared to those with CPH (34.1 %) or CAH 2 a (34.3 %). Averages of pre-treatment serum HCV-RNA amount (copies/50 μl) were $10^{3.55}$ in 13 CRs, $10^{4.56}$ in 17 PRs, and $10^{5.95}$ in 17 NRs. There was a positive correlation between pre-treatment HCV-RNA levels and IFN unresponsiveness. HCV genotyping in 114 patients revealed that HCV type I infection was observed in one, type II in 94, type III in 11, type IV in 6 and mixed (types II and IV) in 2 patients, and their IFN responses (CR/PR/NR) were 0/0/1, 28/26/

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40, 3/5/3, 1/3/2 and 0/1/1, respectively.

Index Terms

chronic hepatitis C, interferon therapy, liver histology, HCV titer, HCV genotype

Hepatitis C virus (HCV) is the causative agent of chronic hepatitis C, which progresses to liver cirrhosis and hepatocellular carcinoma (HCC)[1-3]. Recently, interferon (IFN) administration has been reported effective for chronic hepatitis C[4-6], and the efficacy of IFN therapy for chronic hepatitis C is being established worldwide.

Since January 1992, we have treated 493 patients with chronic hepatitis C by IFN-alpha. In this report, we evaluated the efficacy of IFN therapy and analyzed the factors determining the IFN responsiveness in our own experience of treating patients with chronic hepatitis C by IFN -alpha.

PATIENTS AND METHODS

Patients

A total of 493 consecutive Japanese patients with histologically proven chronic hepatitis received IFN-alpha therapy (Table 1). All patients had anti-HCV (anti-C 100.3 or anti-HCV core) detected by the Ortho Diagnostics RIA and all were negative for serological markers of HBV infection (absence of HBsAg and anti-HBc). All patients gave their written informed consent for participation.

Interferon

Either natural IFN-alpha (nIFN, human lyphoblastoid IFN, Sumitomo Pharmaceuticals Co., Osaka) or recombinant IFN-alpha 2 a (rIFN, Takeda Chem. Ind. Co. Ltd., Osaka) was used at daily doses of 3 million units (MU) or 9 MU, respectively. nIFN or rIFN was given 7 times a week for the initial 2 weeks and subsequently 3 times a week for 22 weeks.

Examinations and follow-up

Liver biopsy was performed before IFN therapy in all patients and liver histology was assessed according to the European classification[7]. Biochemical examination of blood was

Table 1. Characteristics of patients

Variable	Values
Patients	493
Men and woman	344, 149
Average age at start of therapy	49.4
Histology of Liver	
CPH	82
CAH2a	313
CAH2b	98
Treatment	
natural IFN α	302
recombinant IFN α	191

done at 2- to 4-week intervals.

Definition of response to IFN-alpha

Reverse transcription and the polymerase chain reaction (RT-PCR) method detecting HCV -RNA encoding the NS 5 region[8] was used to evaluate IFN reponses. Complete response (CR) was defined as sustained disappearance of HCV-RNA in sera for more than 6 months after the end of IFN treatment. Partial response (PR) was defined as tempory elimination of HCV in IFN treatment period and non-response (NR) as continuing positivity of HCV-RNA throughout the treatment and follow-up periods.

Genotyping of HCV and quantification of HCV-RNA in serum

The genotyping of HCV was performed by the PCR method using type-specific primers that recognize the core region of HCV as reported[9]. HCV-RNA in serum before IFN therapy was quantified by a competitive PCR assay using synthetic mutant RNA with a novel restriction endonuclease (Kpn I) site[10], which was generated by site-directed mutagenesis and in vitro transcription.

Statistics

We used Student's t test for the evaluation of statistical differences.

IFN response

IFN response was evaluated in 342 patients who had been followed more than 6 months after the end of IFN therapy. As shown in Table 2, 97 patients (28.4 %) were classified as CR, 104 (30.4 %) as PR, and 141 (41.2 %) as NR. A statistical difference in CR rate was not observed either between males and females or between nIFN-treated and rIFN-treated patients. Table 3 shows the relation between IFN responses and alanine aminotransferase (ALT) levels. Sustained normalization of ALT levels was obtained in 91 patients in total. Eighty-seven (89.7 %) out of 97 complete responders showed normal ALT levels. Four patients with sustained normal ALT levels were positive for serum HCV-RNA at the last examinations (2 patients

Table 2. IFN responses

	Total	Male	Female	nIFN α	rIFN α
	n=342	n=232	n=110	n=187	n=155
CR	97 (28.4%)	60 (25.9%)	37 (33.6%)	47 (25.1%)	50 (32.3%)
PR	104 (30.4%)	67 (28.9%)	37 (33.6%)	45 (24.1%)	59 (38.1%)
NR	141 (41.2%)	105 (45.3%)	36 (32.7%)	95 (50.8%)	46 (29.6%)

Table 3. Relashion between HCV-RNA detection and ALT level

	Total	Sustained nomalization of ALT level	
	n=342	yes : n=91	no :n=251
CR	97	87	10
PR	104	2	102
NR	141	2	139

showed PR and 2 showed NR).

Rekationship between liver histology and IFN response (Table 4)

There was no difference in CR rate between patients with CPH (34.1 %) and those with CAH 2 a (34.3 %). However, a lower CR rate was observed in patients with CAH 2 b (11.4 %). The NR rates of patients with CPH, CAH 2 a or CAH 2 b were 25.0 %, 34.3 % and 64.8 %, respectively.

Relationship between pre-treatment serum HCV-RNA amount and IFN response

Serum HCV-RNA amount before IFN treatment was examined in 47 patients (13 complete responders, 17 partial responders and 17 non-responders, Fig. 1). The average serum HCV-RNA amount was $10^{3.55}$ copies/50 μl in complete responders, $10^{4.65}$ copies/50 μl in partial responders and $10^{5.97}$ copies/50 μl in non-responders. There were no complete responders who showed over 10^5 copies/50 μl of HCV-RNA amount before IFN treatment. Serum HCV-RNA amounts in NR and PR patients were significantly higher than those in CR patients.

Relationship between pre-treatment HCV-RNA amount in serum and liver histology

The averages of serum HCV-RNA amount were $10^{4.04}$ (copies/50 μl) in 14 patients with CPH, $10^{4.64}$ in 26 with CAH 2 a, and $10^{5.17}$ in 9 with CAH 2 b (Fig. 2). Therer was a tendency for serum HCV-RNA amount to be related to histological severity of liver disorders.

HCV genotype and IFN responsiveness

HCV genotyping was performed in 114 patients, who composed of 32 complete responders, 35 partial responders and 47 non-responders. As shown in Table 5, 94 patients infected with type II of HCV (HCV-II) resulted in 28 CRs, 26 PRs, and 40 NRs. Eleven infected with type III of HCV (HCV-III) resulted in 3 CRs, 5 PRs and 3 NRs. Six patients with type IV of HCV (HCV-IV) in one CR, 3 PRs and 2 NRs. The case infected with type I of HCV (HCV-I) was a non-responder hemophiliac. Both of the two patients infected with HCV-II and IV had experiences

Table 4. Relationship between liver histologies and IFN responses

	CPH n=44	CAH2a n=210	CAH2b n=88
CR	15 (34.1%)	72 (34.3%)	10 (11.4%)
PR	18 (40.9%)	65 (31.3%)	21 (23.8%)
NR	11 (25.0%)	73 (34.3%)	57 (64.8%)

Table 5. Relationship between HCV genotypes and IFN responses

	Type I (n=1)	Type II (n=94)	Type III (n=11)	Type IV (n=6)	Type II+IV (n=2)
CR		28	3	1	
PR		26	5	3	1
NR	1	40	3	2	1

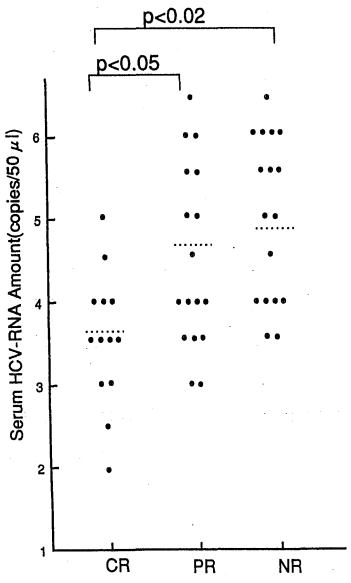

Fig. 1. Relationship between pre-treatment serum HCV-RNA amount and IFN response.

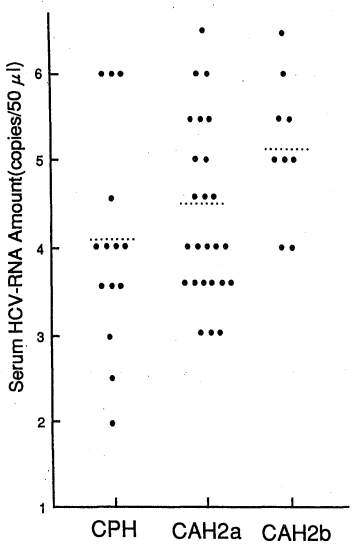

Fig. 2. Relationship between pre-treatment serum HCV-RNA amount and liver histology.

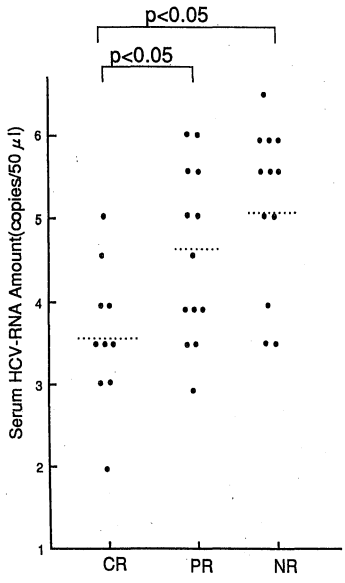

Fig. 3. Relationship between pre-treatment serum HCV-RNA amount IFN response in patients infected with Okamoto type II of HCV.

of massive transfusion and their IFN responses were PR and NR.

DISCUSSION

IFN-alpha is recognized as an useful drug for treatment of chronic hepatitis C. Its efficacy has been mostly evaluated by means of serum ALT levels and improvement of liver histological findings[5,6]. In addition, recent advances of diagnostic techniques, including the application of the PCR for detection of HCV-RNA, allowed us to evaluate IFN responses on the basis of the presence or absence of HCV in serum. In the present study, we evaluated IFN responses virologically using PCR technique and investigated several background factors that might affect IFN responses, such as liver histology, pre-treatment HCV-RNA amount in semum and HCV genotype.

Our first question was whether IFN responses determined by serum HCV-RNA using a RT-PCR method differed from those determined by ALT values. In treating 342 patients with chronic hepatitis C, HCV elimination was obtained in 97 patients and sustained normalization of ALT in 91 patients. Eighty-seven patients showed both HCV elimination and sustained normalization of ALT levels, which corresponded to 89.7% and 95.6% of patients who obtained sustained HCV elimination or ALT normalization, respectively. The evaluations of IFN response by ALT value were almost the same as those determined by HCV-RNA elimination. We therefore conclude that ALT value is clinically sufficient for evaluation of IFN therapy.

We next investigated liver histology, which is thought to be one of the factors determining IFN responses[11,12]. Patients with CAH 2 a responded to IFN better than those with CAH 2 b in the present study. Similar observations were also reported by others[13,14]. The histological severity seemed to correlate to poor responsiveness to IFN therapy. However, we could not find a better responsiveness in patients with CPH than in those with CAH 2 a.

As another factor influencing IFN responsiveness, HCV titer in serum should be considered. We determined pre-treatment HCV-RNA amount by competitive PCR assay. Complete responders had lower pre-treatment HCV-RNA levels than those in partial responders and non-responders. These results suggest that pre-treatment HCV-RNA amount in serum is another important predictor of IFN responses. To exclude the influence of HCV genotypes on IFN responses, we also examined the relation between pre-treatment HCV-RNA amount and IFN response in patients who were infected with an identical genotype of HCV, HCV-II (Fig. 3), and a similar result was obtained. The pre-treatment HCV-RNA amount seemed to be related to histological severity of liver disorder. However, it is noteworthy that there existed some patients with CPH who showed high level of HCV viremia. This may be the reason why we could not obtain a better responsiveness in patients with CPH. In fact, two patients out of three with CPH and high level of pre-treatment HCV-RNA, over 10^6 (copies/50 μl), revealed NR to the therapy. Recently, Lau et al[15] also reported a similar observation and insisted that the level of viremia was not necessarily correlated to liver histology.

It was reported that IFN responsiveness might vary among different HCV genotypes[16-18]. Patients infected with genotype HCV-II are considered to be more resistant to IFN therapy than those with genotype HCV-III or HCV-IV. In the present study, the relationship of viral genotypes to IFN response could not be definitely evaluated, because most of our patients (96 of 118 patients, 84.2 %) were classified to genotype HCV-II. Those with genotype HCV-III or HCV-IV were only 11 (9.7 %) and 6 (5.3 %) patients, respectively. A larger number of patients should be examined for their infected HCV genotypes to determine whether HCV-III or HCV-IV is more sensitive to IFN therapy. It was, however, proven that about one third of patients infected with HCV-II were able to respond well to IFN therapy and obtain complete HCV elimination.

Taken together, in the present study, we observed that liver histology, and pre-treatment HCV-RNA amount in serum could be useful for predicting IFN response.

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