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A Preliminary Study of Retreatment of Chronic Hepatitis C with Interferon

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Abstract Nine patients with chronic hepatitis C virus (HCV) infection and no complete response to the first treatment with natural interferon (IFN)-alpha, were prescribed a second treatment with IFN. Five patients (Group A) with unsustained levels serum alanine aminotransferase (ALT) after the first treatment were administrated the same species of IFN but in a higher dose. The remaining four patients (Group B), with no normalization of ALT throughout the observation period of the first treatment, were administrated IFN-beta. HCV RNA was eliminated in three patients of group A and in two of group B patients during 6 months follow up and ALT reverted to normal levels. These results suggest that retreatment with a higher dose of the same species of IFN-alpha can be effective in case of a relapse and that IFN-beta can be effective for those not responding to IFN-alpha.

Introduction

Hepatitis C virus (HCV) is a major causative agent of chronic non-A, non-B liver disease[3], and chronic liver disease caused by HCV is more common in Japan than is HBV[7)9)]. Because patients with chronic hepatitis C can develop liver cirrhosis and hepatocellular carcinoma[1)13)], early treatment is important.

Interferon (IFN) is effective for patients with chronic hepatitis C as it can reduce serum aminotransferase (ALT) levels and hepatocellular necrosis, control disease activity[10)19)], and eliminate HCV RNA from the sera[2)6)8)11)] However, over 50% of these patients have a relapse after the withdrawal of IFN[4)5)8)16)20)]. There are patients for whom treatment with IFN is ineffective and the dose and duration of treatment, the selection of species of IFN and the combination of other drugs with IFN remain open questions.

We re-treated nine patients with chronic HCV infection previously treated with natural IFN-alpha who did not sustain the elimination of HCV RNA or for whom serum aminotransferase during the follow-up period was not normalized.

Materials and methods

Patients

The criteria for retreatment were a 6-month first treatment with natural IFN-alpha, elevated ALT and detectable HCV RNA after the first treatment, and good tolerance to IFN-alpha. Nine patients meeting these criteria were retreated. After written informed consent was obtained, these Japanese patients were divided into two groups. In five patients (Group A), ALT normalized by the end of the first treatment and HCV RNA was eliminated from the sera of four, but in all five patients HCV RNA re-appeared with elevation of ALT after

Table 1 Patient profile

Case	Age	Sex	Blood Transfusion	Duration of Hepatitis (Yrs.)
A	26	F	(+) 1 year before	1
B	62	M	(−)	20
C	40	M	(−)	4
D	59	F	(+) 37 years before	2
E	55	M	(+) 25 years before	10
F	25	F	(−)	2
G	46	M	(−)	10
H	36	M	(−)	4
I	55	M	(−)	7

Note: "Duration of hepatitis" means the period between the first detection of serum ALT elevation and the initiation of IFN.

the withdrawal of IFN. In four patients (Group B), ALT never normalized and HCV RNA was never eliminated during the observation period.

The second treatment was initiated after the elevation of ALT and re-appearance of HCV RNA. All patients were positive both for antibody to HCV and HCV RNA and were negative for hepatitis B surface antigen, antibody to human immunodeficiency virus and autoantibodies. For a semiquantitive evaluation of the histological changes, we used a numerical scoring system to assess the histological activity index (HAI), as described by Knodell et al[14]. Profiles of the nine patients are shown in Table 1.

Protocol

First treatment: All patients were given intramuscular injections of natural interferon alpha (human lymphoblastoid interferon, Sumypheron, Sumitomo Co., Tokyo, Japan). In four patients, a dose of 3 million units (MU) was given daily for 2 weeks, 3 MU was given three times a week for the next 6 weeks, followed by 1.5 MU three times a week for 16 weeks. In the remaining five, 1 MU was given three times a week for an additional 14 weeks. The total dose given was 210 MU over a 38-week period.

Second treatment: In Group A, five patients were given intramuscular injections of the same species of IFN-alpha as used in the first treatment. A dose of 6 MU was given daily for the first 2 weeks, then three times a week for the next 22 weeks. The total dose given was 480 MU over a 24-week period. In Group B, four patients were given intravenous injections of natural IFN-beta (Human Fibroblast Interferon, Feron, Daiichi Pharmaceutical Co., Tokyo, Japan). A dose of 6 MU was given daily for 6 weeks (Total dose 252 MU).

Methods

Paired blood samples were obtained every two weeks during the therapy, and every 2-4 weeks for at least 3 months before entry and after cessation of the treatment. Each blood sample was tested for complete blood count and routine serum biochemistry. A level of ALT over 40 IU/L was regarded as abnormal.

All serum samples were separated and stored at −20 degree until testing for anti-HCV, HCV RNA, HCV RNA genotype and HCV RNA levels. Anti-HCV (HCV EIA II, Abbott Labo-

ratories, North Chicago, IL, USA), was examined using enzyme linked immunosorbent assay.

Serum HCV RNA was detected by two-stage PCR, using primers from the 5′-noncoding region of the HCV genome: 5′-CTGTGAGGAACTACTGTCTT-3′ (sense; nt 28-47) and 5′-AACACTACTCGGCTAGCAGT-3′ (antisense; nt 229-248) in the first stage, and 5′-TTCACGCAGAAAGCGTCTCTAG-3′ (sense; nt 46-65) and 5′-GTTGATCCAAGAAAGGACCC-3′ (antisense; nt 171-190) in the second stage. The HCV genotypes were determined by two-stage PCR, using universal and type specific primers from the putative C gene of the HCV genome, according to the methods of Okamoto et al[18]. The first stage of PCR was performed with primers consisting of 5′-TGCGCGCGAC (TA) AGGAAGACTTC-3′ (nt 137 to 158 from the HC-J4 isolate, sense) and 5′-ATGTACCCCATGAGGTCGGCGA-3′ (nt 391 to 412 from the HC-J1 isolate, antisense). The second stage of PCR was performed with a sense primer consisting of 5′-AGGAAGACTTCCGAGCGGTC-3′ (nt 148 to 167 from the HC-J1) and a mixture of four type-specific antisense primers. Antisense primers specific for the four HCV types were 5′-TGCCTTGGGGATAGGCTGAC-3′ (nt 185 to 204, type Ⅰ), 5′-GAGCCATCCTGCCCACCCCA-3′ (nt 272 to 291, type Ⅱ), 5′-CCAAGAGGGACGGGAACCTC-3′ (nt 302 to 321, type Ⅲ) and 5′-ACCCTCGTTTCCGTACAGAG-3′ (nt 251 to 270, type Ⅳ). They were deduced from regions in the putative C gene where the sequences were preserved only by a group of isolates, with minor variations. The expected sizes of the products by the second stage of PCR were 57 bp (type Ⅰ), 144 bp (type Ⅱ), 174 bp (type Ⅲ), and 123 bp (type Ⅳ), respectively.

HCV RNA in serum was quantified by a competitive RT-PCR assay[12] using synthetic mutant HCV RNA, which is restricted by EcoRI in the 5′-noncoding region. HCV RNA from patients was mixed in each tube with the diluted mutant HCV RNA (102-7 copies/50μL) and competitive PCR with 5′-noncoding region was carried out. The amplified products were then restricted by EcoRI and were analyzed by electrophoresis, mutant HCV RNA demonstrated in 106 bp and 112 bp and HCV RNA from patients in 218 bp. Size of the PCR products from patients was compared with that of the diluted mutant HCV RNA. The titer of circulating HCV RNA was defined as log 10 (copy numbers of HCV RNA per fifty microliter of serum).

Results

Figure 1 shows the clinical course of the five patients in Group A during the first and second treatment with natural IFN-alpha. The serum ALT levels in all patients normalized and HCV RNA was eliminated in four by the end of the second treatment. The serum ALT in three patients remained at normal levels with the elimination of HCV RNA: in the remaining two it re-elevated with detectable HCV RNA after the second treatment. HCV RNA in case E was not eliminated from the serum with either first or second treatments.

Figure 2 shows the clinical course of the four patients in Group B. By the end of the second treatment, the serum ALT levels had elevated in all patients and HCV RNA was eliminated in two. During the 6 months follow-up, the serum ALT levels normalized in these two patients. Finally, five (three in Group A and two in Group B) (55.6%) of nine patients responded to the second IFN treatment and the remaining four (44.4%) did not respond. In two patients who responded to IFN-beta, ALT normalized after the elimination of HCV RNA, while in three patients who responded to IFN-alpha normalization and HCV RNA elimination occurred simultaneously. Three of four patients in whom HCV RNA was transitionally eliminated in the first treatment responded completely to a higher dose of the same species of IFN-alpha.

Those HCV RNA levels under 4 responded to

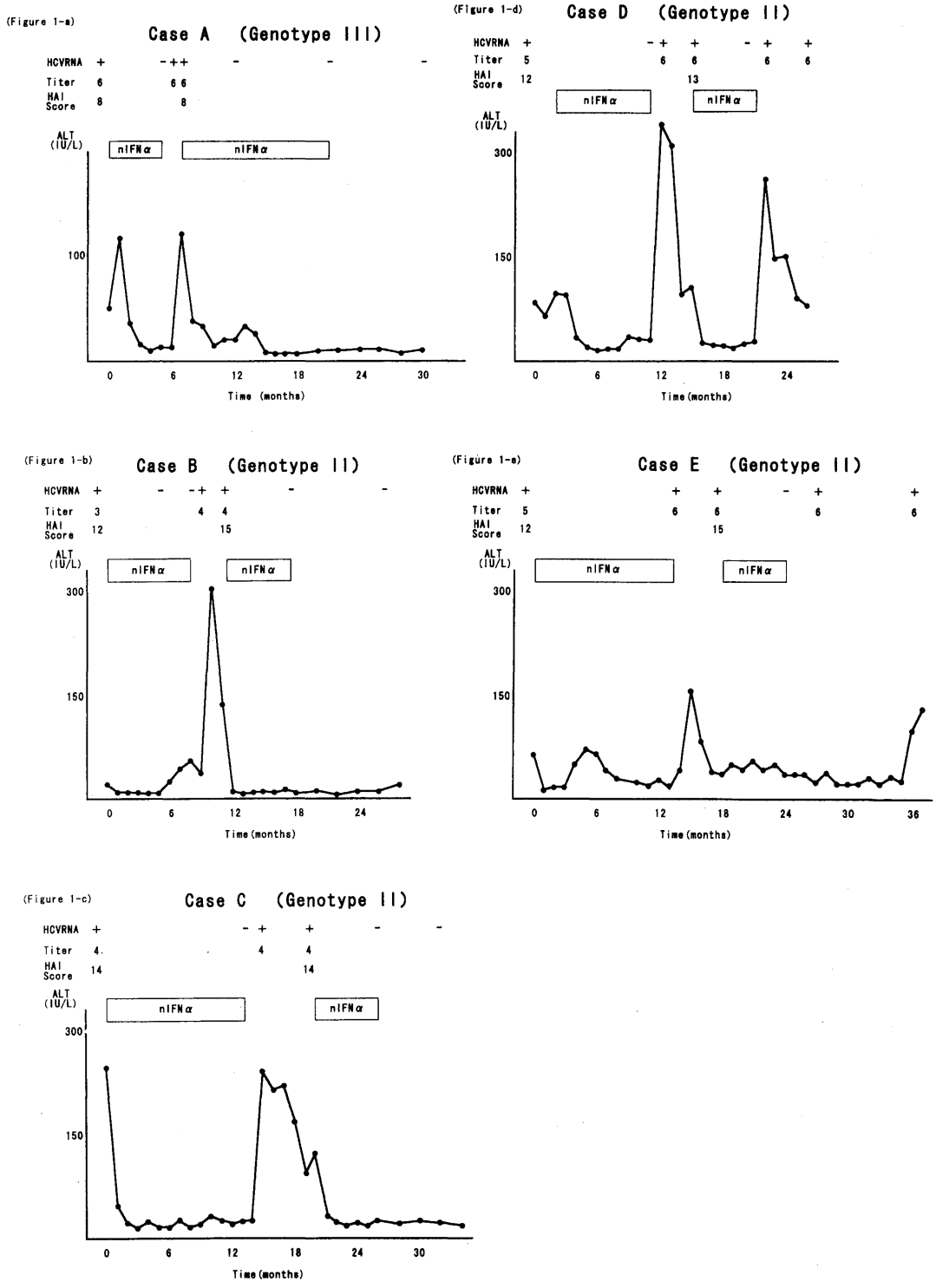

Fig. 1 Clinical course throughout the first and second treatment of Group A patients. HCV RNA titer was defined as log 10 (copy numbers of HCV RNA per 50 microliter of serum.

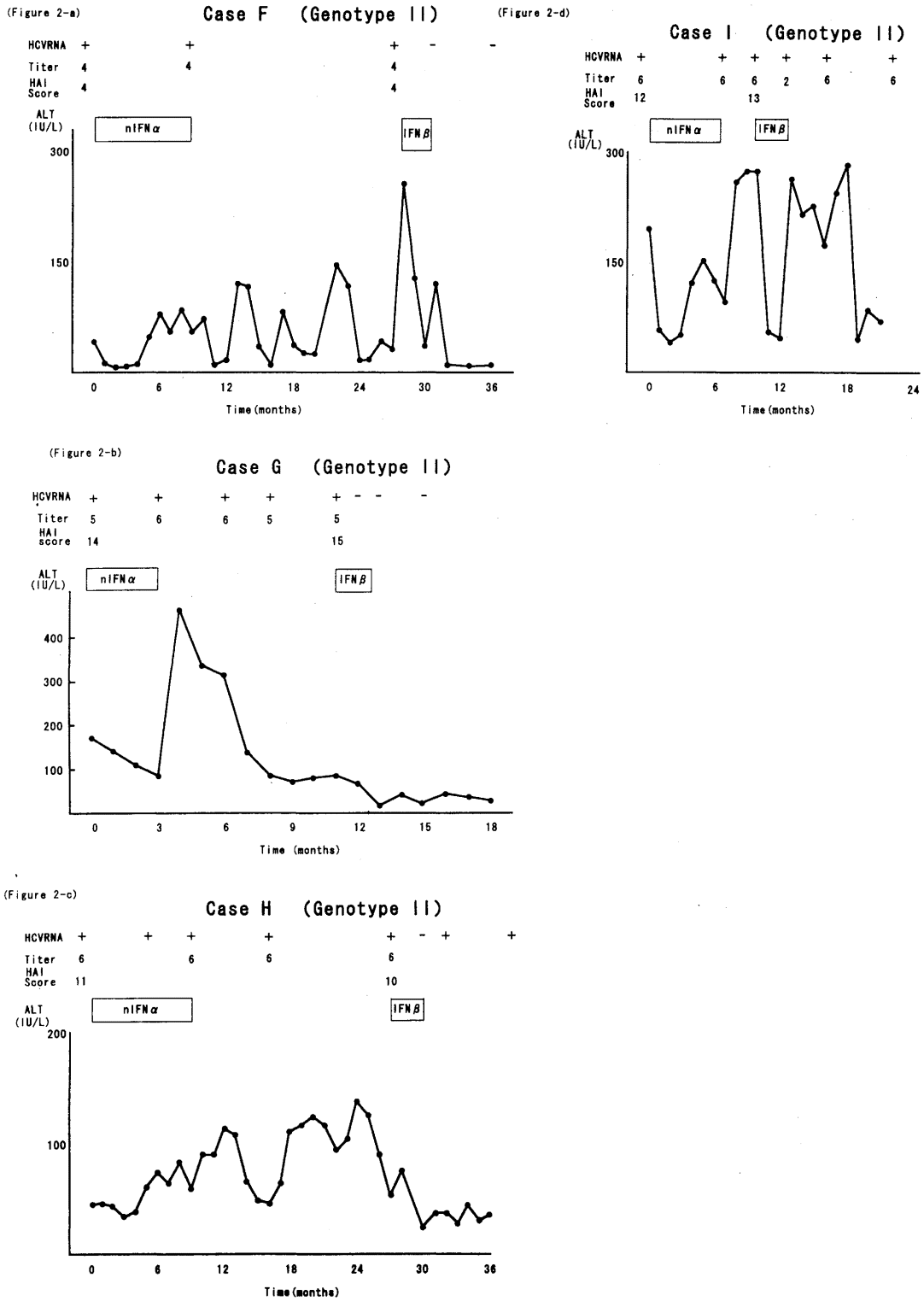

Fig. 2 Clinical course throughout the first and second treatment of Group B patients.

the second treatment. Three of seven with HCV RNA levels over 4 responded, one of them had genotype III HCV RNA. The serum ALT levels in six patients were higher when measured before initiation of the second treatment than the levels measured before the first treatment: three responded to the second treatment (Table 2). The HAI scores in five patients determined before the second treatment were increased as compared to those taken before the first treatment: three responded to the second treatment.

Discussion

There are few reports concerning the retreatment of hepatitis C with IFN. Marcellin et al[17] reported that patients with chronic hepatitis C who had not responded to the first treatment did not respond to a second treatment, if given the same schedule and dose of IFN-alpha. Of our patients who did not respond to the first treatment with IFN-alpha 60% had a complete response to the second treatment with a higher dose of the same species of IFN-alpha. However, in these patients responding to the second treatment there was a transient elimination of HCV RNA with the first treatment. The second treatment with IFN-beta was effective for whom HCV RNA was not eliminated in the first treatment with IFN-alpha. Kobayashi et al.[15] reported that IFN-beta was as effective as IFN-alpha for treatment of patients with chronic hepatitis C. As IFN-beta is five times more expensive than IFN-alpha in Japan, it is not cost effective to use IFN-beta for the first treatment; however, it can be used effectively for patients who did not respond to IFN-alpha in the first or second treatment.

Another interesting finding of this study is that the normalization of ALT occurred after the elimination of HCV RNA with IFN-beta treatment, yet normalization occurred simultaneously with IFN-alpha treatment[2)8)10)]. Injury to hepatocytes caused by IFN-beta may exceed that caused by IFN-alpha.

Pretreatment parameters for the prediction

Table 2 Comparison of characteristics of parients before the first and second treatments with interferon

Case	Source and dose of IFN (MIU)		HCV Genotype	HAI Score		HCV RNA[a)] titer		s-ALT (IU/L)	
	First	Second		First	Second	First	Second	First	Second
A	HLBI 16800	HLBI 48000	3	8	8	6	6	50	120
B	HLBI 21000	HLBI 48000	2	12	15	4	3	70	180
C	HLBI 21000	HLBI 48000	2	14	14	4	4	245	120
D	HLBI 16800	HLBI 48000	2	17	17	6	6	103	134
E	HLBI 16800	HLBI 48000	2	12	13	6	6	120	118
F	HLBI 21000	IFN-β 25200	2	4	6	6	7	43	256
G	HLBI 16800	IFN-β 25200	2	14	15	6	6	283	85
H	HLBI 21000	IFN-β 25200	2	11	10	6	6	61	90
I	HLBI 21000	IFN-β 25200	2	12	13	6	6	195	272

a)HCV RNA titer was defined as log 10 (copy numbers of HCV RNA per 50 microliter of serum.
HLBI=Human Lymphoblastoid Interferon; HAI=Histology Activity Index;
ALT=Alanine Aminotransferase;

of response to IFN were absence of cirrhosis and a younger age. We have data that a high level of HCV RNA and genotype II are markers of an unfavorable response to IFN as determined in a multiple logistic regression analysis (unpublished data). In the present study findings, three of five patients responding to the second treatment fit the predictive markers (two had low levels of HCV RNA and the other was of genotype III). However, the elevation of ALT and the deterioration of liver histology after the first treatment had no apparent influence on the effectiveness of the second treatment.

In conclusion, a second treatment with a higher dose of the same species of IFN-alpha was effective for patients with chronic hepatitis C with a transient elimination of HCV RNA by the end of the first treatment, and IFN-beta was effective for retreatment of patients for whom the virus was not eliminated throughout the course of the first treatment.

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(和文抄録)

C型慢性肝炎に対するインターフェロン再投与例の検討

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慢性C型肝炎で初回のインターフェロン（IFN）治療にて治癒しなかった9人の患者に対してIFNによる再治療を行った．初回治療終了時にALTが正常化したがその後再上昇した5人の患者（Group A）には初回と同種のIFNを増量して投与した．初回治療でALTの正常化がみられなかった4人の患者（Group B）にはIFNの種類を変更して治療した．再治療後Group Aのうち3人，Group Bのうち4人で治療終了6カ月後でもHCV RNAの持続陰性化とALTの正常化がみられていた．よって再発した例ではより高用量のIFNによる，そして初回治療時に反応しない例ではIFNの種類の変更による治療が有効であると考えられた．