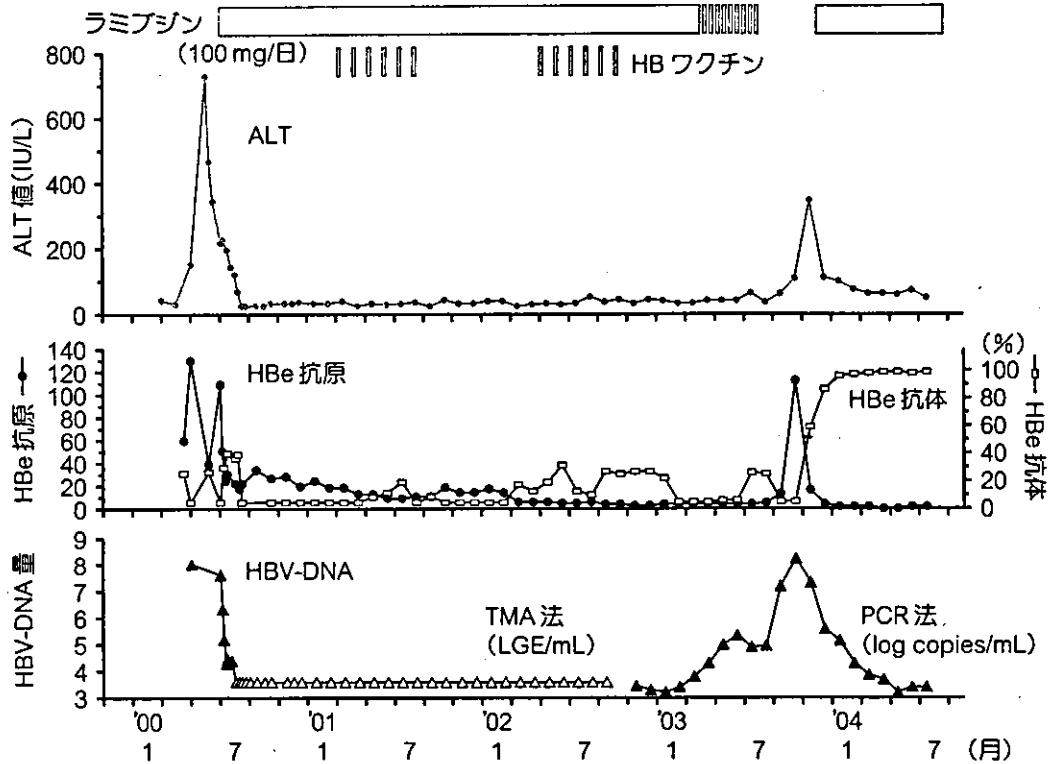


## II. 症例 1. HBe 抗原陽性 B 型慢性肝炎症例

症例 7. ラミブジン投与中止後に肝炎の再燃を起こし、ラミブジン再投与により改善した症例

### 臨床経過



2003年7月のラミブジン内服中止後に肝炎の再燃を認めたが、同年11月から投与を再開し、セロコンバージョンを認めた。

HBV-DNA量の測定方法は経過中に変更したが、TMA法での△は測定感度以下を示している。

下、HBe抗体上昇の傾向を示しており、ラミブジン内服がセロコンバージョンへの流れを加速させたものと考えられた。

インターフェロンやラミブジン投与でセロコンバージョンが得られなかった難治例に対して、ラミブジン休薬による肝障害の再燃を利用してセロコンバージョンを達成させた。本例のように、ラミブジン投与後にウイルス学的には反応を示すもののセロコンバージョンを得られないため長期投与を余儀なくされる症例は少なからず認められ、投与期間の長期化に伴う変異ウイルス出現のリスクを考慮した場合のジレンマとなっている。比較的若年で肝線維化の高度でない症例に対しては、本例のように、意図的にラミブジン投与を中止し、リバウンド後の再投与により改善させる方法も検討可能であると考えられる。

## 症例 18. ラミブジン投与により肝機能の改善がみられた肝硬変症例 (YMDD 変異ウイルス未出現)

髙 修平

患者背景 61歳 女性

診断名	B型肝炎肝硬変	罹病期間	17年	入院 or 外来	外来
既往歴	21歳：十二指腸潰瘍, 43歳：尾骨骨折				
家族歴	妹：B型慢性肝炎				
生活歴	ビール 500 mL/日, 喫煙歴なし				

○前治療薬：なし

病 歴

1985年に尾骨骨折をきたした際にHBs抗原陽性を指摘され、慢性肝炎の診断を受け、近医で治療を受けていた。1991年に当科を初診したが、以後、HBe抗体陽性のままHBV-DNA量の変動を認め、慢性肝炎から肝硬変への進展がみられた。さらに、2001年に肝障害の増強とともに肝予備能の低下傾向を認めたため、2001年9月にラミブジンの投与を開始した。

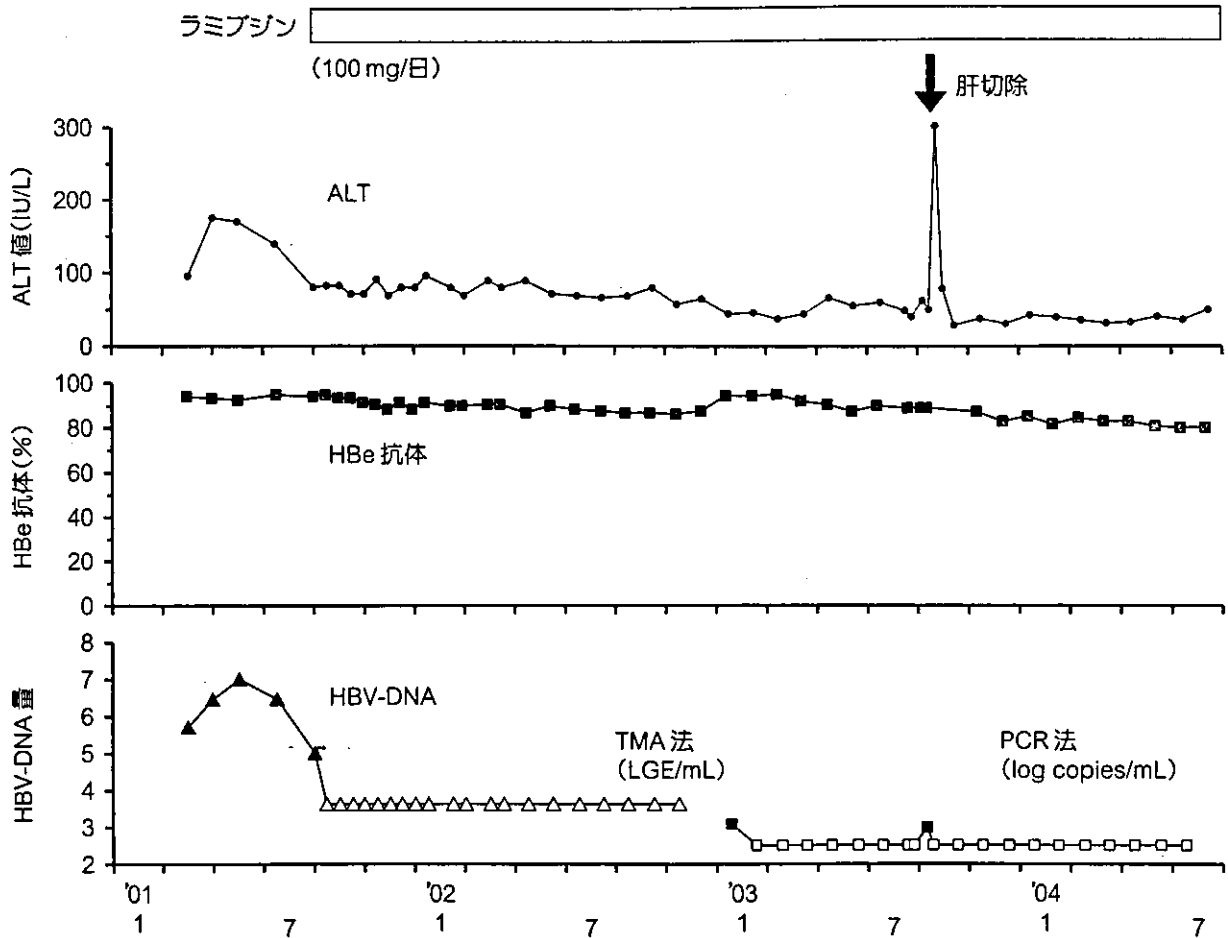
経 過

治療開始時点で、トランスアミナーゼの低下傾向を認め、HBV-DNA量も減少傾向を示していたが、治療開始後、ウイルス量は急速に減少し、ALT値も改善した。投与後約6カ月間で、アルブミン値は3.1から3.9(g/dL)、コリンエステラーゼ値は130から164(IU/L)、ヘパプラスチンテストは33.2から49.7(%)へと、肝機能の改善傾向を認めた。現在まで投与開始から3年が経過したが、HBe抗体は陽性を持続しHBV-DNA量も低値で推移しており、YMDD変異ウイルスの出現はみられていない(臨床経過図1, 2)。

なお、経過中の2003年7月に腹部CTで肝腫瘍を発見され、肝細胞癌の診断にて、同年9月17日に肝S4+S8の部分切除術を施行した。摘出標本の病理組織学的所見では、背景肝は完成した肝硬変であるが炎症細胞浸潤は乏しく、活動性の低い状態であった(図1)。手術直後は、一時的にALT値の上昇を認めたが、肝予備能の低下は軽度で、順調な回復を示した。

症例 18. ラミブジン投与により肝機能の改善がみられた肝硬変症例  
(YMDD 変異ウイルス未出現)

臨床経過図 1



2001年9月からラミブジン内服を開始し、肝障害の改善がみられた。2003年9月に肝癌に対する肝切除術を施行され、一時的な肝障害を認めたが、回復は良好であった。HBV-DNA量は継続して低値を維持している。

HBV-DNA量の測定方法は経過中に変更したが、TMA法の△、PCR法の□は、それぞれの方法での測定感度以下を示している。

考 察

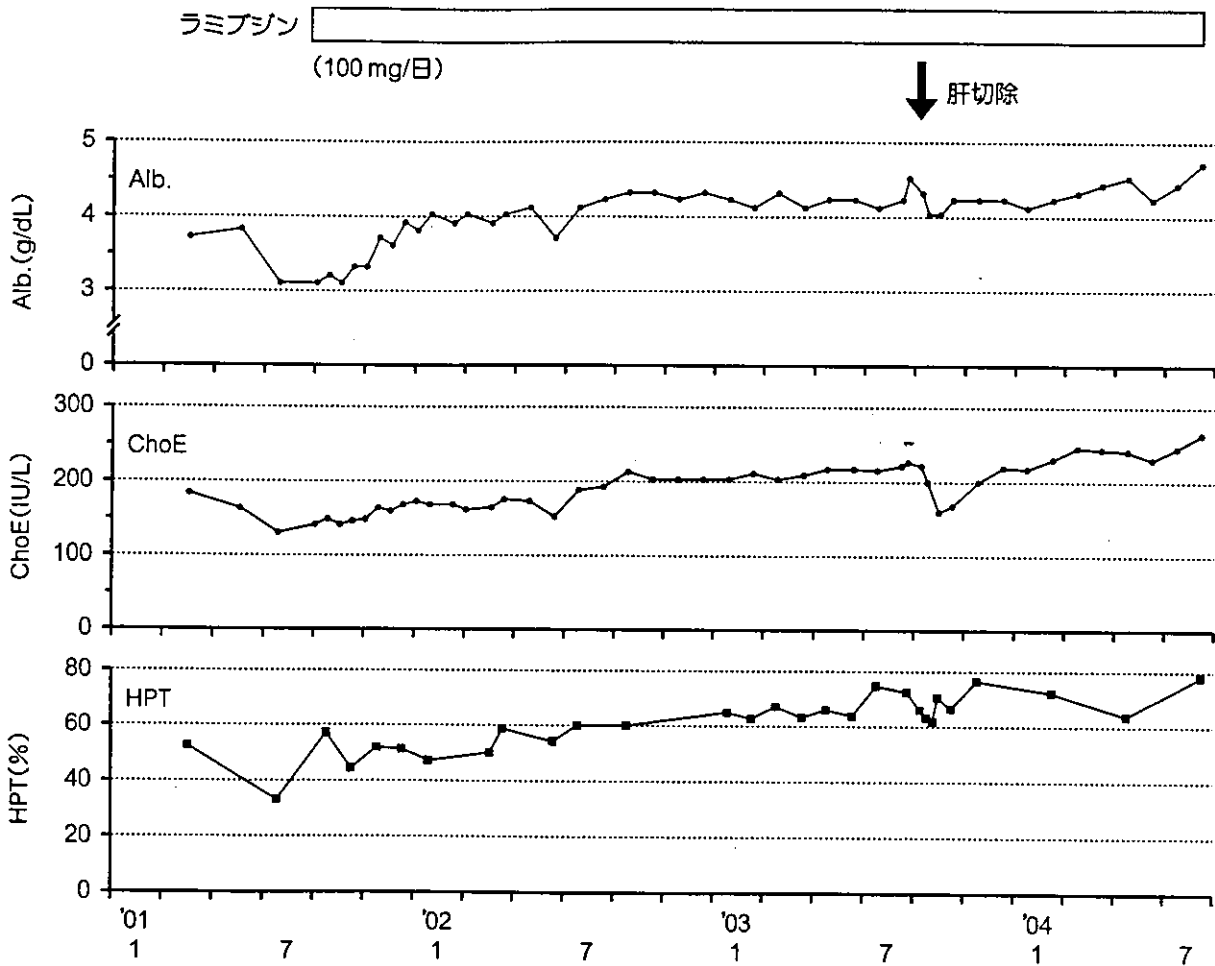
本症例の治療経過やポイントを整理すると、

- 1) HBe抗体陽性で肝炎が持続する症例では、ALT値はHBV-DNA量に連動することが多い。したがって、このような症例に対しては、長期的にウイルス量を低値に維持し肝炎の鎮静化を図ることが肝病変の進展抑制の意味で重要である。

## II. 症例 4. HBe 抗体陽性 B 型肝炎硬変症例

症例 18. ラミブジン投与により肝機能の改善がみられた肝硬変症例  
(YMDD 変異ウイルス未出現)

○臨床経過図 2

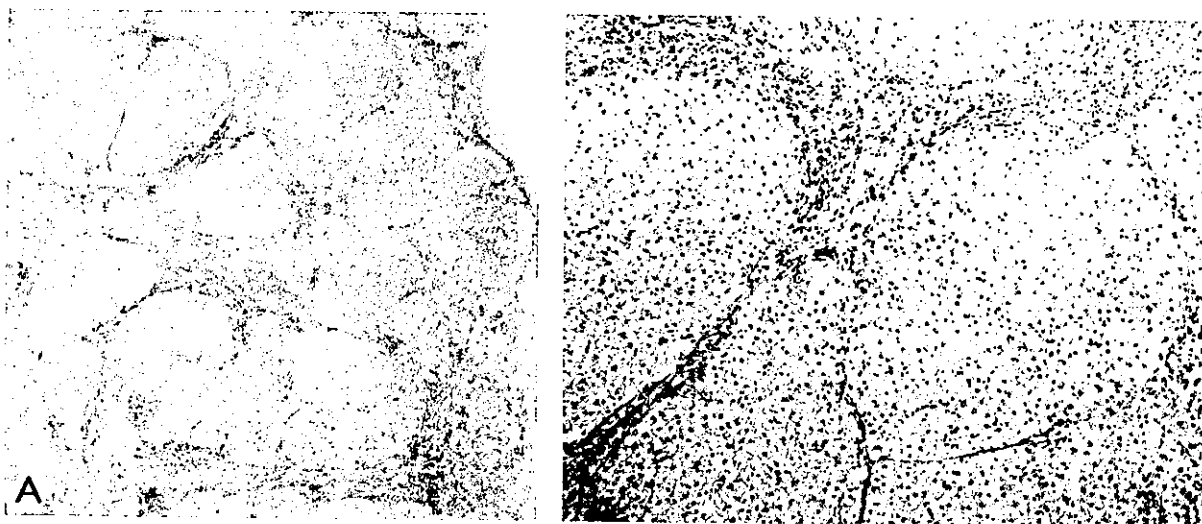


ラミブジン投与後、肝予備能は順調に改善傾向を認めた。肝切除後、一時的に肝予備能の低下を認めたが、その後の回復は良好であった。

- 2) 特に、肝硬変症例では、肝不全への進展を抑止する意味でも有用である。本症例において、投与開始約2年後に肝切除が施行された際の背景肝組織では炎症所見は乏しく、有効性が確認された。
- 3) 3年間のラミブジン継続投与の段階ではYMDD変異ウイルスは出現していない。治療開始時点でe抗体陽性症例やHBV-DNA量低値症例では変異ウイルスの出現は比較的低率で、

症例 18. ラミブジン投与により肝機能の改善がみられた肝硬変症例  
(YMDD 変異ウイルス未出現)

○図1 2年後の肝組織像



ラミブジン治療開始の約2年後に施行された肝切除時の肝組織像 (A:弱拡大, B:強拡大)。完成された肝硬変の所見であるが、肝炎の活動性は軽度であった。

出現時期も、e抗原陽性やDNA量高値例に比べて遅い傾向がある。

4) 肝癌を合併した場合には、肝癌の進展と肝予備能を総合的に判断して、手術、肝動脈塞栓術、ラジオ波焼灼術、リザーバー動注化学療法などの中から治療法を選択するが、肝予備能の維持は、十分な癌治療の達成のためにも重要となってくる。本症例では肝癌発見以前からのラミブジン内服により肝予備能が改善していたため、安全に手術を施行する事が可能であった。ラミブジン内服開始時のChild-PughスコアではB(7点)であったが、手術時にはA(5点)に改善していた。

肝炎活動性を持続する肝硬変症例に対しては、肝不全への進展抑制の意味でラミブジン治療は有用である。さらに、肝癌を合併した場合には、最も望ましい肝癌治療法の選択、維持のためにも重要な治療となる。

## Changes in virus loads and precore mutations in chronic hepatitis B patients treated with 4 weeks of daily interferon alfa-2a therapy

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### Abstract

Interferon (IFN) alfa-2a was administered to 23 patients with chronic hepatitis B daily for 4 weeks and the relation between the efficacy of the treatment and changes in total hepatitis B virus (HBV) DNA and precore mutant levels was investigated. At 6 and 12 months after the completion of IFN therapy, 39.1% (9/23) and 36.8% (7/19) of patients, respectively, showed alanine transaminase (ALT) normalization; 31.3% (5/16) and 50.0% (7/14), respectively, became negative for HBe-antigen (HBeAg); and 42.1% (8/19) and 41.2% (7/17), respectively, became undetectable for HBV DNA. All 18 of the patients who were positive for HBeAg at baseline nevertheless had the precore mutation. The level of precore mutant as a proportion of the total HBV DNA level was constant at baseline, and 3 and 6 months after the completion of therapy. Thus, the investigation showed that in chronic hepatitis B, the precore mutation occurs at a constant proportion beginning in the HBeAg-positive phase, and IFN therapy inhibits the growth of the wild-type and precore mutant viruses equally.

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**Keywords:** Chronic hepatitis B; rIFN $\alpha$ -2a; HBV DNA; Precore mutant

### 1. Introduction

Drugs that have been used to treat chronic hepatitis B include interferon (IFN), propagermanium, and steroids. Recently, lamivudine has been introduced and its use in combination with IFN has attracted interest. IFN therapy was first reported by Greenberg et al. [1] in 1976, whose work shows that IFN inhibits viral growth. In the US and Europe, IFN monotherapy generally consists of long-term administration of 5–10MU per day three times per week for 4–6 months [2,3]. In Japan, in 1986, the National Health Insurance coverage established 4 weeks as the standard treatment period. Consequently, in the present study, rIFN alfa-2a was administered daily for 4 weeks at a dose of 9MU per day for the first 3 days and 18MU per day thereafter.

The efficacy of IFN therapy is estimated by seroconversion from HBe-antigen (HBeAg) to HBe-antibody (HBeAb),

undetectable response for hepatitis B virus (HBV) DNA, and normalization of alanine transaminase (ALT). Factors reported to be associated with response to IFN therapy are the baseline levels of HBV DNA and ALT [2,3]. Moreover, the clinical significance of infection with the precore mutant virus, which does not produce HBeAg, has recently drawn attention. We therefore, quantitatively analyzed precore mutant levels and examined the changes in these levels with IFN monotherapy.

### 2. Materials and methods

The subjects were 23 patients with chronic hepatitis B, 16 males and 7 females, with a mean age of  $36.3 \pm 9.8$  years. Eighteen of the patients were positive for HBeAg and five were negative. Although all 23 patients were positive for HBV DNA in the polymerase chain reaction (PCR) assay, two patients were below detection limits by the bDNA probe assay. The precore mutant level was not less than  $10^7$  copies/ml in 12 patients and less than  $10^7$  copies/ml in 11

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Table 1  
Baseline characteristics of patients

Patients number	Sex	Age	Grading <sup>a</sup>	Staging <sup>a</sup>	Interval from prior IFN (Month)	ALT (IU/l)	HBeAg (index)	HBeAb (%)	HBVDNA bDNA-p (Meq/ml)	HBVDNA PCR (copy/ml)	Precore mutant (copy/ml)
1	Female	35	–	–	38	287	0.8	96.4	96	8 × 10 <sup>7</sup>	8 × 10 <sup>7</sup>
2	Male	46	A1	F1	–	67	0.8	95.1	<0.7	5 × 10 <sup>3</sup>	<100
3	Female	52	A2	F1	10	310	179.8	0	1900	3 × 10 <sup>8</sup>	3 × 10 <sup>8</sup>
4	Female	29	–	–	24	304	3.6	57.8	3.4	3 × 10 <sup>6</sup>	3 × 10 <sup>6</sup>
5	Male	34	A1	F0	–	74	53.5	0	44	3 × 10 <sup>7</sup>	8 × 10 <sup>6</sup>
6	Female	22	A1	F1	–	259	407.7	0	78	3 × 10 <sup>7</sup>	3 × 10 <sup>7</sup>
7	Female	34	A1	F1	7	116	385.4	0	190	7 × 10 <sup>7</sup>	4 × 10 <sup>7</sup>
8	Male	43	A2	F2	14	206	113.3	0	1800	4 × 10 <sup>8</sup>	7 × 10 <sup>8</sup>
9	Male	56	A3	F2	–	148	7.2	58.0	1.6	9 × 10 <sup>5</sup>	9 × 10 <sup>5</sup>
10	Male	33	A1	F0	–	142	271.9	0	350	1 × 10 <sup>8</sup>	1 × 10 <sup>5</sup>
11	Male	33	–	–	41	87	292.2	0	150	3 × 10 <sup>7</sup>	3 × 10 <sup>7</sup>
12	Male	40	A2	F0	–	995	451.8	0	2100	4 × 10 <sup>8</sup>	4 × 10 <sup>8</sup>
13	Male	28	A1	F1	–	42	0.8	82.1	710	8 × 10 <sup>8</sup>	8 × 10 <sup>8</sup>
14	Male	44	A2	F2	49	57	1.8	17.2	2.8	5 × 10 <sup>6</sup>	8 × 10 <sup>5</sup>
15	Female	30	A2	F1	–	211	183.4	0	3800<	8 × 10 <sup>8</sup>	8 × 10 <sup>8</sup>
16	Female	27	A2	F2	–	87	9.2	23.3	<0.7	9 × 10 <sup>4</sup>	5 × 10 <sup>3</sup>
17	Male	36	–	–	10	448	231.8	0	25	1 × 10 <sup>7</sup>	1 × 10 <sup>6</sup>
18	Male	33	A3	F3	7	44	5.2	54.7	1	3 × 10 <sup>6</sup>	1 × 10 <sup>6</sup>
19	Male	27	A1	F2	5	184	53.9	0	400	3 × 10 <sup>7</sup>	3 × 10 <sup>7</sup>
20	Male	58	A3	F3	13	367	0.8	87.6	74	3 × 10 <sup>7</sup>	3 × 10 <sup>7</sup>
21	Male	26	A1	F3	8	115	172.8	0	1000	2 × 10 <sup>8</sup>	1 × 10 <sup>8</sup>
22	Male	28	A2	F0	10	338	174.7	0	490	1 × 10 <sup>7</sup>	7 × 10 <sup>6</sup>
23	Male	42	–	–	96	191	2.2	78.6	2.1	1 × 10 <sup>5</sup>	5 × 10 <sup>3</sup>

<sup>a</sup> Five cases were not measured.

patients, 1 of whom had a precore mutant level of less than 10<sup>2</sup> copies/ml. Fourteen patients had previously received IFN therapy for intervals from 5 to 96 months, during which they had been administered 477MU daily for 4 weeks. Baseline ALT was 34 to 66 IU/l in three patients and not less than 67 IU/l in 20 patients. Eighteen patients underwent liver biopsy, of whom four had a fibrosis score of F0, six a score of F1, five a score of F2, and three a score of F3 (Table 1).

rIFN alfa-2a was initially administered at a dose of 9MU per day for three consecutive days and 18MU per day for the subsequent 25 days (total dose, 477MU). Excluded were patients who had received an antiviral agent or immunomodulator within 3 months before the study; those who had received an injectable agent containing glycyrrhizin/cysteine/glycine or shosaiko-to (Chinese herbal medicine) within 1 month before the study; and those with a white blood cell (WBC) count of less than 3000/mm<sup>3</sup> or a platelet count of less than 100,000/mm<sup>3</sup>.

The virological tests performed were the total amount of HBV DNA, using a bDNA probe assay (Quantiplex, Chiron) and competitive polymerase chain reaction assay (nested-PCR, Otsuka Assay), and the HBV precore mutant levels, using a quantitative mutation-site specific polymerase chain reaction assay (PCR-MSSA, Otsuka Assay). Using PCR-MSSA assay, precore point mutation (G→A, 83rd base of precore region) was examined using a mutation-trapped oligonucleotide primer, which yields a polymerase chain reaction amplification product only with precore mutants and within the detection limits of 10<sup>2</sup> to 10<sup>9</sup> copies/ml [4]. Each

measurement was performed immediately before treatment initiation, at treatment completion, and 6 months after treatment completion. HBeAg and HBeAb levels were measured immediately before treatment initiation, at treatment completion, and 3, 6, and 12 months after treatment completion. They were measured by radioimmunoassay (RIA), and a cutoff index higher than 2.1 for HBeAg was judged to be positive, and an inhibition percent higher than 50 for HBeAb was judged to be positive. Liver histology findings were assessed according to the Knodell histologic activity index [5] and the Desmet scoring system [6].

The efficacy of the treatment was evaluated at its completion and at 6 and 12 months after completion according to ALT normalization and loss of HBeAg and HBV DNA.

The statistical analysis was performed using Fisher's exact test and the Wilcoxon 2-sample test.

### 3. Results

#### 3.1. Efficacy

The rate of patients with normalized ALT levels was 4.3% (1/23) at treatment completion, 39.1% (9/23) at 6 months after completion, and 36.8% (7/19) at 12 months after completion. Although all measurement rates changed during the follow-up, there were many cases with normalized ALT levels after the treatment completion. Of the patients who were positive for HBeAg at baseline, the rate of patients who

Table 2  
The rate of biochemical and virological response

	At treatment completion	3 months after treatment completion	6 months after treatment completion	12 months after treatment completion
ALT normalized	4.3% 1/23	34.8% 8/23	39.1% 9/23	36.8% 7/19
HBeAg lost <sup>a</sup>	38.9% 7/18	29.4% 5/17	31.3% 5/16	50.0% 7/14
HBV DNA cleared <sup>b</sup>	47.6% 10/21	40.0% 8/20	42.1% 8/19	41.2% 7/17

Reduction of the number of patients during the follow-up is caused by without patient's consent.

<sup>a</sup> Five patients were excluded because negative at study initiation.

<sup>b</sup> Two patients were excluded because undetectable at study initiation.

became negative for HBeAg was 38.9% (7/18) at treatment completion, 31.3% (5/16) at 6 months after completion, and 50.0% (7/14) at 12 months after completion. Thus, the highest negative rate was at 12 months after the treatment completion. The rate of patients who became undetectable for HBV DNA (bDNA probe assay) was 47.6% (10/21) at treatment completion, 42.1% (8/19) at 6 months after completion, and 41.2% (7/17) at 12 months after completion. A rate of more than 40% undetectable was maintained after the treat-

ment completion. The inability to obtain consent resulted in a reduction in the number of patients followed (Table 2).

### 3.2. Efficacy according to patient baseline characteristics

Examination of baseline patient characteristics, ALT normalization, and loss of HBeAg and HBV DNA at 6 months after treatment completion revealed a trend toward greater efficacy with respect to the rate of ALT normalization and

Table 3  
Efficacy according to baseline characteristics of patients

Features		n	6 Months after treatment completion		
			ALT normalized (n = 23)	HBeAg lost <sup>a</sup> (n = 16)	HBV DNA cleared <sup>b</sup> (n = 19)
Sex	Male	16	25.0% (4/16)	20.0% (2/10)	38.5% (5/13)
	Female	7	71.4% (5/7)	50.0% (3/6)	50.0% (3/6)
Age	<40	15	40.0% (6/15)	25.0% (3/12)	38.5% (5/13)
	40≤	8	37.5% (3/8)	50.0% (2/4)	50.0% (3/6)
Prior IFN therapy	Yes	14	35.7% (5/14)	40.0% (4/10)	53.8% (7/13)
	No	9	44.4% (4/9)	16.7% (1/6)	16.7% (1/6)
Staging	F0,F1	10	50.0% (5/10)	28.6% (2/7)	25.0% (2/8)
	F2,F3	8	37.5% (3/8)	20.0% (1/5)	66.7% (4/6)
	Non-perform	5	20.0% (1/5)	50.0% (2/4)	40.0% (2/5)
ALT (IU/ml)	67≤	20	35.0% (7/20)	33.3% (5/15)	37.5% (6/16)
	34–66	3	66.7% (2/3)	0% (0/1)	66.7% (2/3)
HbeAg (index)	100–1000	11	27.3% (3/11)	22.2% (2/9)	33.3% (3/9)
	2.1–100	7	42.9% (3/7)	42.9% (3/7)	50.0% (3/6)
	<2.1	5	60.0% (3/5)	–	50.0% (2/4)
HBeAb (%)	50–100	8	62.5% (5/8)	50.0% (2/4)	42.9% (3/7)
	0–50	15	26.7% (4/15)	25.0% (3/12)	41.7% (5/12)
HBV DNA (Meq./ml)	100≤	11	27.3% (3/11)	25.0% (2/8)	33.3% (3/9)
	0.7–100	10	40.0% (4/10)	42.9% (3/7)	50.0% (5/10)
	<0.7	2	100% (2/2)	0% (0/1)	–
HBV DNA (copies/ml)	10 <sup>7</sup> –10 <sup>9</sup>	16	31.3% (5/16)	27.3% (3/11)	35.7% (5/14)
	10 <sup>2</sup> –10 <sup>7</sup>	7	57.1% (4/7)	40.0% (2/5)	60.0% (3/5)
	<10 <sup>2</sup>	0	–	–	–
Precore mutant (copies/ml)	10 <sup>7</sup> –10 <sup>9</sup>	12	41.7% (5/12)	37.5% (3/8)	45.5% (5/11)
	10 <sup>2</sup> –10 <sup>7</sup>	10	30.0% (3/10)	25.0% (2/8)	37.5% (3/8)
	<10 <sup>2</sup>	1	100% (1/1)	–	–

<sup>a</sup> Five patients were excluded because negative at study initiation.

<sup>b</sup> Two patients were excluded because undetectable at study initiation.



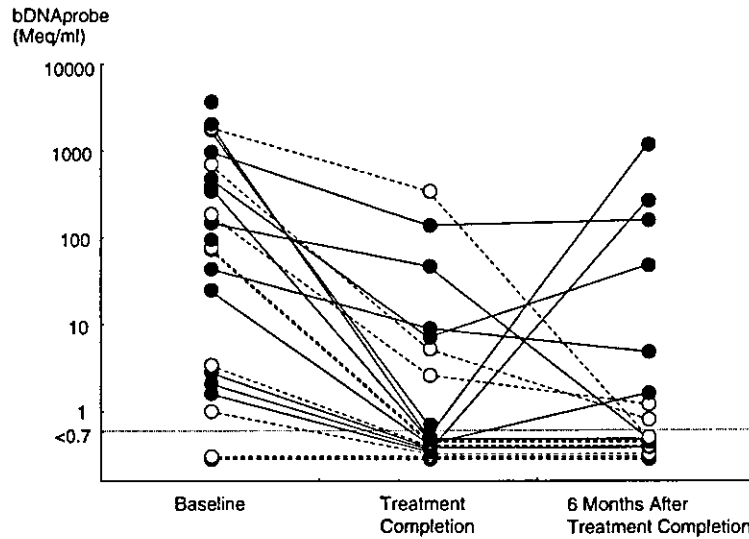


Fig. 1. Changes in Serum HBV DNA levels on interferon (IFN) therapy. And normalization of ALT in each patients at 6 months after the treatment completion. HBV DNA levels were significantly lower in patients with normalized ALT than with abnormal ALT at 6 months after the treatment completion. Open circles with dotted lines showed the changes in serum HBV DNA levels in normalized alanine transaminase (ALT) at 6 months after treatment completion, and closed circles with solid lines showed the abnormal alanine transaminase (ALT) at the same time. It was performed by the bDNA probe assay, since the changes in total hepatitis B virus (HBV) DNA levels were clearer than by the polymerase chain reaction (PCR) assay.

loss of HBeAg and HBV DNA in female patients than in male patients. Moreover, patients who had undergone previous IFN therapy showed greater efficacy, as indicated by the negative rate for HBeAg and HBV DNA, than patients who had not undergone previous therapy. In addition, the lower the baseline viral load, the greater was the efficacy with respect to the rate of ALT normalization and loss of HBeAg and HBV DNA. However, there were no significant differences between baseline characteristics and efficacy (Table 3).

3.3. Efficacy based on changes in viral markers

Regardless of its level at baseline, HBV DNA tended to decrease from the initiation of IFN therapy to its comple-

tion. After treatment completion, this decrease continued in five patients, while HBV DNA levels increased in the remaining patients. Two of the patients who exhibited more than 100Meq/ml of the virus at treatment completion had ALT normalization at 6 months after treatment completion; conversely, seven patients who had less than 100Meq/ml of the virus or were undetectable for the virus at treatment completion did not show ALT normalization at 6 months after treatment completion. Thus, there was no relation between the virus level at treatment completion and efficacy at 6 months after completion. However, HBV DNA levels at 6 months after treatment completion were significantly lower in patients with normalized ALT than with abnormal ALT at the same time (the ALT normalization rate of positive HBV

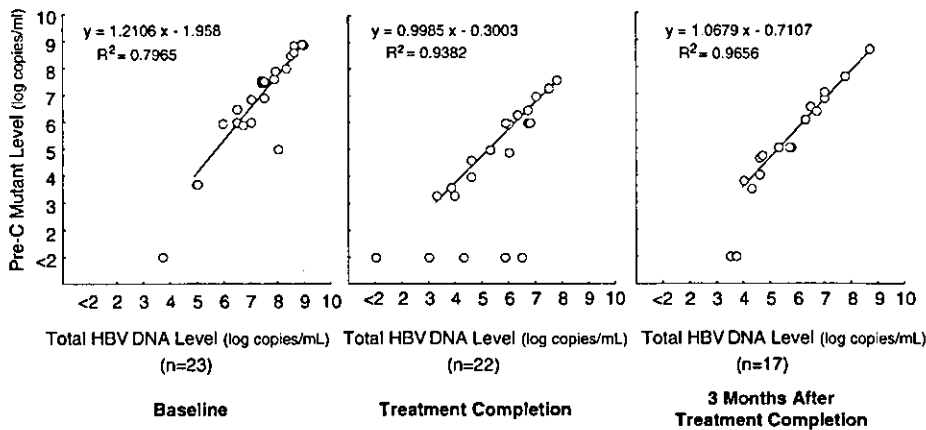


Fig. 2. Total HBV DNA Levels and Precore Mutant Levels on interferon (IFN) Therapy. Total hepatitis B virus (HBV) DNA and precore mutant were measured in: 23 patients at baseline, 22 patients at the treatment completion and 17 patients at 3 months after the treatment completion. Correlation coefficients are shown at each point. From the point of sensitivity of detection limit during the treatment, hepatitis B virus (HBV) DNA was performed by the polymerase chain reaction (PCR) assay.

DNA patients was 25%, and 70% for negative HBV DNA patients;  $P = 0.0359$ ). It performed by the bDNA probe assay, since the changes in total HBV DNA levels were clearer than by the PCR assay (Fig. 1).

At baseline, only one patient had a precore mutant level of less than  $10^2$  copies/ml and was thus considered to have the wild-type virus. A correlation was seen between the total HBV DNA level and the precore mutant level at baseline, with no consistent trend seen between the proportion of precore mutant in relation to total HBV DNA. A correlation between total HBV DNA and precore mutant level was also seen at treatment completion and 3 months after completion, with no trend seen between the proportion of precore mutant at these timepoints. At all timepoints, the slope of the curve was equal, and no change was seen in the mutant proportion. However, there were exceptions in four patients: only the precore mutant level decreased to the limit of detection at treatment completion, indicating that inhibition of precore mutant growth exceeded inhibition of wild-type growth. From the point of sensitivity of detection limit during the treatment, HBV DNA was performed by the PCR assay (Fig. 2).

#### 4. Discussion

Although no drugs have shown adequate efficacy in chronic hepatitis B, lamivudine has recently been introduced. Lamivudine has potent anti-HBV activity, but long-term lamivudine treatment frequently produces the YMDD mutation and increases HBV DNA and ALT levels [7]. As treatment discontinuation in this case may produce a rebound effect and result in acute exacerbation of the hepatitis, strategies such as continued lamivudine therapy with concurrent IFN use have been adopted [8]. Combined therapy with lamivudine and IFN reportedly increases the negative rate for HBeAg [9].

The first results of 4 weeks of IFN monotherapy in Japan were reported in 1983 by Matsumura et al. [10]. Since then, there have been occasional reports on topics such as the virological and immunological changes seen during IFN monotherapy, but there have been few reports on the efficacy of IFN therapy. The criteria for evaluating the efficacy of IFN therapy are not clearly defined. Generally, negative response for HBeAg, seroconversion to HBeAb, and normalization of ALT are assessed. Recently, however, the results of clinical studies using liver carcinogenesis or survival as the endpoint have been reported. Factors such as IFN therapy, age, histological progression in the liver, and post-treatment negative for HBeAg, HBV DNA, and HBsAg and normalization of ALT, play significant roles in efficacy after treatment completion [11–13].

In the present study, treatment with rIFN alfa-2a for 4 weeks resulted in, at 6 months and 12 months after treatment completion, normalization of ALT and almost the same undetectable HBV DNA rate, however the negative HBeAg

rate rose from about 30 to 50%. Thus, in this study, treatment with rIFN alfa-2a did not show its effectiveness at each time-point. It will be necessary to perform long-term follow-up observations of these patients, using liver carcinogenesis or survival as the endpoint.

The close relation between HBV mutation and disease type has been shown, and there have been numerous studies on this topic. Mutation at nucleotide 1896 of the precore region is considered particularly important, because it is thought to be related to the pathophysiology of fulminant and other hepatitis. In this study, we evaluated the frequency of the occurrence of precore mutants using MSSA for the detection of point mutations at the 83rd base in the precore region for the mutant HBV genome. MSSA can be used for the detection of  $10^2$  copies/ml of precore mutants in the presence of  $10^7$  copies/ml of wild-types, its sensitivity is considered to be at least 0.001%. With the use of this MSSA, even if only wild-type genomes are present, precore mutant-type can be identified on electrophoresis [4]. There have been numerous reports in the US and Europe indicating that this precore mutant is resistant to IFN [14,15]. In Japan, however, IFN efficacy in patients positive for HBeAb was first reported by Muraoka et al. [16], and since then other groups have also reported inhibition of HBV growth and ALT normalization with IFN therapy [17,18]. Possible factors in this discrepancy include the fact that, in the US and Europe, negative responses, not only for HBeAg and HBV DNA, but also for HBsAg, are the objectives of treatment and baseline characteristics such as the period of infection and HBV genotype differ greatly. Therefore, an examination of the response to IFN in which there is uniformity with respect to these factors is needed. In the present study, the total HBV DNA level and precore mutant level were correlated before and after IFN treatment. Among the 18 patients who were positive for HBeAg at baseline, none was negative for the precore mutant. Thus, in chronic hepatitis B, the precore mutation occurred at a constant proportion beginning from the HBeAg-positive phase, and IFN therapy inhibited growth of the wild-type virus and the precore mutant virus equally in most of the patients. In some patients, inhibition of the precore mutant growth exceeded inhibition of the wild-type growth; there were no cases in which wild-type growth was inhibited. Shindo et al. [19] reported that the precore wild-type and mutant have similar sensitivities to IFN. However, because they used the restriction fragment length polymorphism (RFLP) assay [20] to determine mutant virus levels and did not perform a quantitative examination, and because the IFN was administered intermittently (three times per week for 17 weeks) and the efficacy rate was 26.1% (6/23 patients; defined as showing seroconversion to HBeAb, loss of HBV DNA and normalization of ALT), the results of their study cannot be adequately compared with those of our investigation.

In an investigation in patients with type B cirrhosis, Ikeda et al. [21] reported high rates of carcinogenesis when the precore mutant is present at high concentrations. They

further report that high precore mutant concentrations correlate with a high total HBV DNA level in such patients, indicating that the inflammation associated with the hepatitis is severe, and acts to indirectly promote carcinogenesis. In addition, it is reported that a primary infection by HBV with a gene mutation contributes to the infection becoming fulminant and severe [22]. However, it is also reported that mutation of the precore region during the natural course of chronic hepatitis B is related to quiescence of the hepatitis and a decrease in the virus level [23].

The time required to reach the true endpoint of the present study, carcinogenesis or survival, makes it difficult to provide conclusions regarding whether the hepatic lesions in patients will progress or whether the hepatitis will become quiescent and the patients will become asymptomatic carriers. However, it is already evident that IFN treatment inhibits the growth of both the wild-type and precore mutant viruses seen in chronic hepatitis B and that it is also effective in patients who are positive for HBeAg and have a predominance of the wild-type virus.

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## Circulating soluble Fas levels in patients with hepatitis C virus infection and interferon therapy

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**Background.** The clinical relevance of the circulating soluble form of the Fas-Receptor (sFas) was investigated in patients with hepatitis C receiving type 1 interferon (IFN) therapy. **Methods.** sFas was quantified by enzyme-linked immunosorbent assay in 66 hepatitis C virus (HCV) carriers and 30 HCV-naive or previously infected controls. The levels were then monitored during enhanced treatment with type 1 IFNs in 15 chronic hepatitis C patients. **Results.** The HCV carriers had high levels of sFas compared with controls ( $3.8 \pm 1.3$  vs  $2.7 \pm 0.8$  ng/ml;  $P < 0.001$ ). sFas levels in patients with chronic HCV infection were directly related to serum alanine aminotransferase levels ( $r = 0.440$ ;  $P < 0.001$ ) and the histological grade ( $r = 0.403$ ;  $P = 0.019$ ). Among necroinflammatory reactions, only piecemeal necrosis showed a correlation with sFas levels ( $r = 0.556$ ;  $P = 0.001$ ). Pretreatment sFas levels, however, were not predictive of therapeutic outcomes. A sustained virological response to enhanced IFN therapy showed a relation to only the pretreatment HCV load. Interestingly, circulating sFas was upregulated when IFN- $\beta$  was administered at short intervals (3MU/every 12h). This upregulation was accompanied by parallel aminotransferase elevation, which was observed regardless of a virological response. **Conclusions.** sFas elevation, in parallel with the severity of liver injury, suggests the possible upregulation of hepatic Fas expression and the Fas-mediated pathway in both HCV- and type 1 IFN-induced liver injury. The essential function of sFas to protect hepatocytes against Fas-mediated liver injury was not evident in these clinical settings.

**Key words:** hepatitis C, type 1 interferon, apoptosis, soluble Fas

### Introduction

Fas-Receptor/Fas ligand-mediated apoptosis of liver cells plays a significant role in the pathogenesis of hepatitis C. Hepatic upregulation of Fas was found to be correlated with more severe inflammation caused by ongoing hepatitis C virus (HCV) infection.<sup>1,2</sup> Parallel activation of T lymphocytes expressing Fas ligand was detected in liver-infiltrating mononuclear cells, allowing transduction of the apoptotic death signal to Fas-bearing hepatocytes.<sup>3</sup> Interferon (IFN)- $\alpha$  and IFN- $\beta$ , members of the type 1 IFN family, are currently used for the treatment of chronic hepatitis C. Hepatic injury in patients treated with systemic administration of type 1 IFNs remains an unresolved problem. A recent study showed that apoptosis may also be involved in type 1 IFN-induced hepatotoxicity.<sup>4</sup>

Fas-Receptor (APO-1/CD95) has a single membrane-spanning domain and is expressed in hepatocytes. An alternatively spliced soluble form of the Fas-Receptor (sFas) without the transmembrane-spanning domain is produced by Fas-expressing hepatocytes,<sup>5,6</sup> and upregulation of Fas expression in hepatocytes contributes to the increase in serum sFas levels. It is of interest that sFas may serve to bind Fas ligand on cytotoxic T lymphocytes, thereby minimizing Fas-mediated liver injury.<sup>7-9</sup> However, the clinical relevance of circulating sFas is not yet fully understood in relation to chronic hepatitis C and type 1 IFN therapy. sFas elevation has been reported in chronic HCV infection,<sup>10</sup> but controversy remains. Possible upregulation of sFas during IFN therapy has also been shown, based on sFas levels within 24h after IFN administration,<sup>11</sup> but the data are limited. To further our understanding of sFas, we studied serum sFas levels in patients with HCV infection as compared with HCV-naive subjects or previously infected controls. sFas levels were then monitored in the course of treatment with type 1 IFNs, and the results were examined in relation to liver injury.

## Patients and methods

### Patients

The subjects were 66 patients with chronic HCV infection (39 men and 27 women; age, 27–80 years [median, 59 years]) and 30 controls without ongoing HCV infection (21 men and 9 women, age 26–73 years [median, 56 years]). No significant difference was seen in sex ( $P = 0.426$ ) or age ( $P = 0.761$ ) distribution between the two groups. The 66 HCV carriers had been persistently positive for serum HCV RNA for more than 6 months. During the entire follow-up, none of the patients displayed any confounding etiology of liver disease, such as hepatitis B virus, autoimmune markers, or alcohol abuse (>25 g/day). Sera were obtained from all patients, and liver biopsy specimens could be obtained from 35 patients at the time of serum collection. After enrollment, 15 of the chronic hepatitis C patients (10 men and 5 women, age 33–63 years [median, 45 years]) were assigned to two different regimens of 4-week enhanced IFN induction therapy. Eight patients (group A) were treated with a high dose of IFN- $\alpha$ 2a (Roferon-A; Nippon Roche, Tokyo, Japan; 9MU/daily for 4 weeks, followed by 9MU/thrice weekly; total dose, 720–1206 MU [median, 941 MU]). The remaining 7 patients (group B) received a high dose of IFN- $\beta$  (Feron; Toray Industries, Tokyo, Japan; 3MU/twice a day for 4 weeks, followed by 6MU/thrice weekly; total dose, 276–636 MU [median, 384 MU]). Using stored serum samples obtained at 0, 1, 2, 4, and 8 weeks after initiation of the induction therapy, we investigated changes in serum alanine aminotransferase (ALT) activity, HCV RNA, and sFas levels.

Sera were also obtained from the 30 control subjects. The controls consisted of 8 healthy individuals without serum HCV RNA and HCV antibody, 8 serum HCV RNA-negative but HCV antibody-positive healthy individuals with no history of IFN therapy, and 14 nonviremic HCV antibody-positive patients who had been successfully treated with IFN therapy for chronic hepatitis C. Patients in the last group had remained negative for serum HCV RNA for 0.7–8.0 years (median, 4.0 years) after the end of previous IFN therapy. All serum samples were stored at  $-80^{\circ}\text{C}$  without thawing until use. The study was approved by the local Research Ethics Committee, in accordance with the 1975 Declaration of Helsinki, and all the patients provided written informed consent.

### Laboratory testing

HCV antibody was tested with a third-generation enzyme-linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Tokyo, Japan). Serum HCV RNA

was detected by a polymerase chain reaction assay (Amplicor HCV Monitor; Roche Diagnostics, Tokyo, Japan) and quantified using a branched DNA (bDNA) assay (Quantiplex HCV-RNA; Chiron, Emeryville, CA, USA). HCV RNA-positive serum samples were subjected to HCV genotyping with the genotype-specific NS4 antibody assay (Immucheck-HCV Gr; International Reagent, Kobe, Japan), which is based on a comparison between antibody responses to the two genotype-specific NS4 antigens (C14-1 and C14-2).

### Detection of sFas

Serum sFas levels were measured by ELISA (Medical and Biological Laboratories [MBL], Nagoya, Japan).<sup>12,13</sup> Serum sFas was first captured by polyclonal antibody to an intracellular sequence of human Fas (KDITSDSENSNFRNEIQSLV), and then detected by monoclonal antibody to an extracellular epitope (KCRCKPNFFC). Thus, the assay is theoretically expected to detect a complete sFas molecule composed of the extracellular and intracellular domains of Fas-Receptor, but is unlikely to detect incomplete cleaved forms of Fas-Receptor, which can be released following the destruction of Fas-expressing cells. In order to quantify the sFas levels, human Fas (GenBank accession no. CAA45250) was expressed in WR19L-12a cells, and standard Fas protein was purified from the membrane fraction. The assay was performed according to the manufacturer's instructions. In brief, a 96-well plate coated with the polyclonal antibody was incubated with 100  $\mu\text{l}$  of fivefold diluted serum samples and standard Fas protein (0–2 ng/ml) at room temperature for 1 h. After thorough washing, the plate was further incubated with the peroxidase-conjugated monoclonal antibody at room temperature for 1 h. The plate was then washed, and a solution containing tetramethyl benzidine was added. After 30-min incubation, the reaction was stopped, and the optical density at 450 nm was measured. All assays for sFas were done in duplicate, and the concentration of sFas was determined from the standard curve. High levels of sFas were quantified using diluted serum samples.

### Histological evaluation

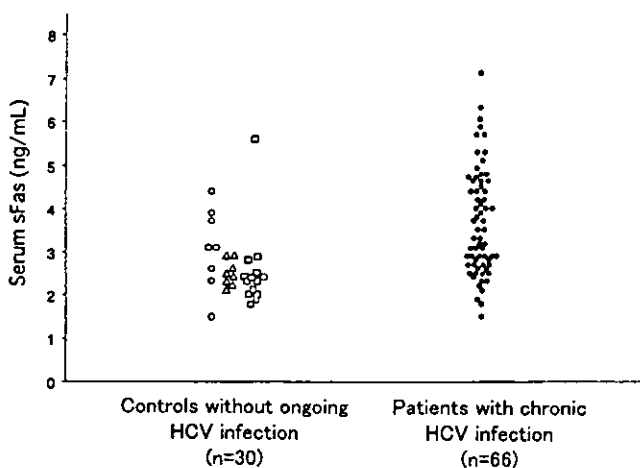
Liver biopsy specimens were fixed in formalin and embedded in paraffin for routine staining with hematoxylin-eosin. All specimens were examined by the same experienced pathologist, who was unaware of the biochemical, serological, and virological data. Biopsy specimens were semiquantitatively evaluated by the histological activity index described by Knodell et al.<sup>14</sup>

### Statistical analysis

All data values are expressed as means  $\pm$  SD. For numeric variables, group comparisons were performed by the Wilcoxon nonparametric test. The  $\chi^2$  test or Fisher's exact test was used for categorical variables. Correlations between the variables were calculated using Spearman rank order correlations. A value of  $P < 0.05$  (two-tailed) was considered to indicate significance. For multivariate analysis, multiple logistic regression analysis, with a stepwise procedure, was performed with the computer program, SAS 8.02 (SAS Institute, Cary, NC, USA).

### Results

Figure 1 shows serum sFas levels in relation to the presence of ongoing HCV infection. The 66 chronic HCV carriers had significantly higher levels of sFas ( $3.8 \pm 1.3$  ng/ml) than the 30 controls without ongoing HCV infection ( $2.7 \pm 0.8$  ng/ml;  $P < 0.001$ ). In the control group, remote history of HCV infection exerted no impact on sFas levels. Serum sFas levels were  $2.5 \pm 0.3$  ng/ml for the serum HCV RNA-negative but HCV antibody-positive healthy individuals who were spontaneously cleared of HCV. The levels were compatible

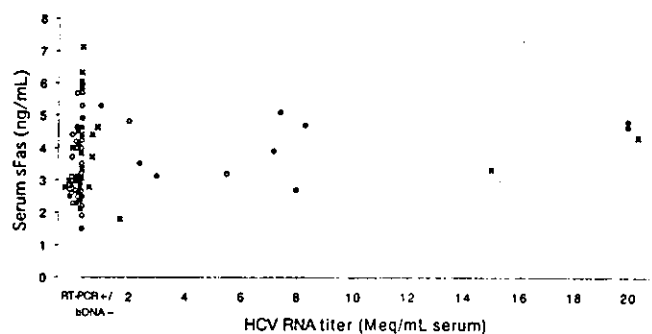


**Fig. 1.** Serum sFas levels in 66 patients with chronic hepatitis C virus (HCV) infection were compared with those in 30 controls without ongoing HCV infection ( $3.8 \pm 1.3$  vs  $2.7 \pm 0.8$  ng/ml;  $P < 0.001$ ). The controls consisted of 8 healthy individuals without serum HCV RNA and HCV antibody (open circles), 8 serum HCV RNA-negative but HCV antibody-positive healthy individuals without interferon (IFN) therapy (open triangles), and 14 patients who had remained negative for serum HCV RNA for 0.7–8.0 years (median, 4.0 years) after the end of IFN therapy for chronic hepatitis C (open squares). The levels of serum soluble Fas-Receptor (sFas) were  $3.1 \pm 0.9$ ,  $2.5 \pm 0.3$ , and  $2.5 \pm 0.9$  ng/ml for these three control groups, respectively, with no significant difference

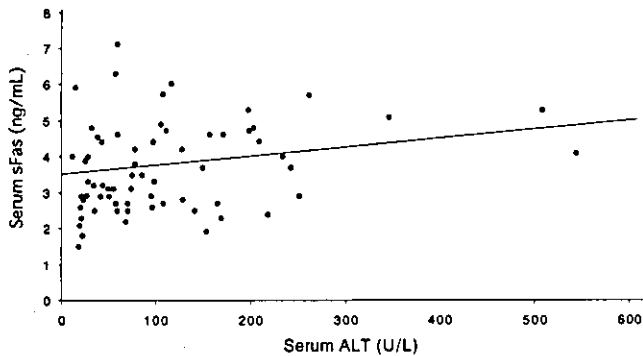
with those for healthy individuals with no HCV markers ( $3.1 \pm 0.9$  ng/ml). sFas levels were equally low in chronic hepatitis C patients who had been clear of serum HCV RNA for 0.7–8.0 years (median, 4.0) after the end of IFN therapy ( $2.5 \pm 0.9$  ng/ml). No relation of sFas levels with time after IFN therapy was evident in these patients. No correlation was seen between sFas levels and age in the chronic HCV carriers ( $r = 0.007$ ;  $P = 0.954$ ) or the control subjects ( $r = 0.103$ ;  $P = 0.579$ ).

The elevation of circulating sFas levels in chronic HCV infection was investigated in relation to virological, biochemical, and histological characteristics. In the 66 chronic HCV carriers, sFas levels had no relation to HCV replicative levels, assessed by serum HCV RNA titers (Fig. 2). This was also the case when the correlation was analyzed for each HCV genotype. sFas levels were  $3.6 \pm 1.2$  ng/ml for the 19 patients infected with genotype 1,  $3.6 \pm 1.1$  ng/ml for the 22 patients with genotype 2, and  $3.8 \pm 1.4$  ng/ml for the remaining 25 patients with undetermined genotype(s), with no significant differences among the values. On the other hand, a significant correlation was found between serum sFas levels and ALT activity ( $r = 0.440$ ;  $P < 0.001$ ; Fig. 3). In 35 chronic HCV carriers, the circulating sFas levels were also correlated with liver histological findings (Fig. 4). A significant correlation was revealed between sFas levels and the total grading score ( $r = 0.403$ ;  $P = 0.019$ ), whereas no correlation was evident between sFas levels and the staging score ( $r = 0.321$ ;  $P = 0.057$ ). As for correlations with necroinflammatory scores, sFas levels showed a correlation with piecemeal necrosis ( $r = 0.556$ ;  $P = 0.001$ ), but not with lobular necrosis and inflammation ( $r = 0.224$ ;  $P = 0.192$ ) or portal inflammation ( $r = 0.316$ ;  $P = 0.066$ ).

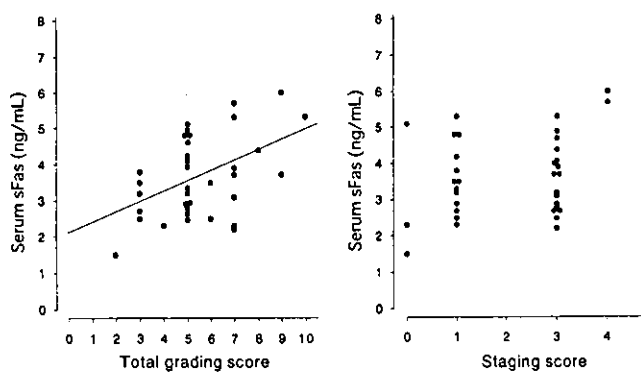
Changes in serum sFas levels during 8 weeks of enhanced induction treatment with type 1 IFNs were



**Fig. 2.** Relation of serum sFas levels with serum HCV RNA titers and HCV genotypes in the 66 patients with chronic HCV infection. Serum sFas levels had no relation to serum HCV RNA titers and HCV genotypes. Solid circles, patients with genotype 1; open circles, patients with genotype 2; crosses, patients with undetermined genotype



**Fig. 3.** Relationship between serum sFas levels and serum alanine aminotransferase (ALT) levels in the 66 patients with chronic HCV infection ( $r = 0.440$ ;  $P < 0.001$ )



**Fig. 4.** Relationship between serum sFas levels and histological findings in 35 patients with chronic HCV infection. Serum sFas levels showed a correlation with the total grading score ( $r = 0.403$ ;  $P = 0.019$ ), but not with the staging score ( $r = 0.321$ ;  $P = 0.057$ )

investigated in 15 chronic hepatitis C patients (Fig. 5). The pretreatment viral load ranged from less than 0.5 to 20.0 Meq/ml (median, 3.0 Meq/ml). Genotypes 1 and 2 were found in 9 and 6 patients, respectively. Of the 8 patients treated with IFN- $\alpha$ 2a (group A), serum HCV RNA was cleared during the treatment in 7 patients. In these virological responders, no significant change was seen in serum sFas levels at weeks 1, 2, 4, and 8 as compared with the baseline level ( $3.7 \pm 0.9$  ng/ml). Thus, HCV clearance was not accompanied by a decrease in serum sFas levels. Serum ALT activity was normalized in 4 virological responders, but remained elevated in the other 3. In group B, the 7 patients received enhanced induction treatment with IFN- $\beta$ . A virological response was achieved during the treatment by 6 patients. Despite HCV clearance, the virological responders showed an increase in sFas levels after the initial intensive treatment (3 MU/twice a day for 4 weeks;  $4.5 \pm 0.9$  at baseline vs  $7.1 \pm 1.3$  ng/ml at week 4;  $P = 0.018$ ). The levels returned to the baseline after the subsequent treatment (6 MU/thrice weekly for 4

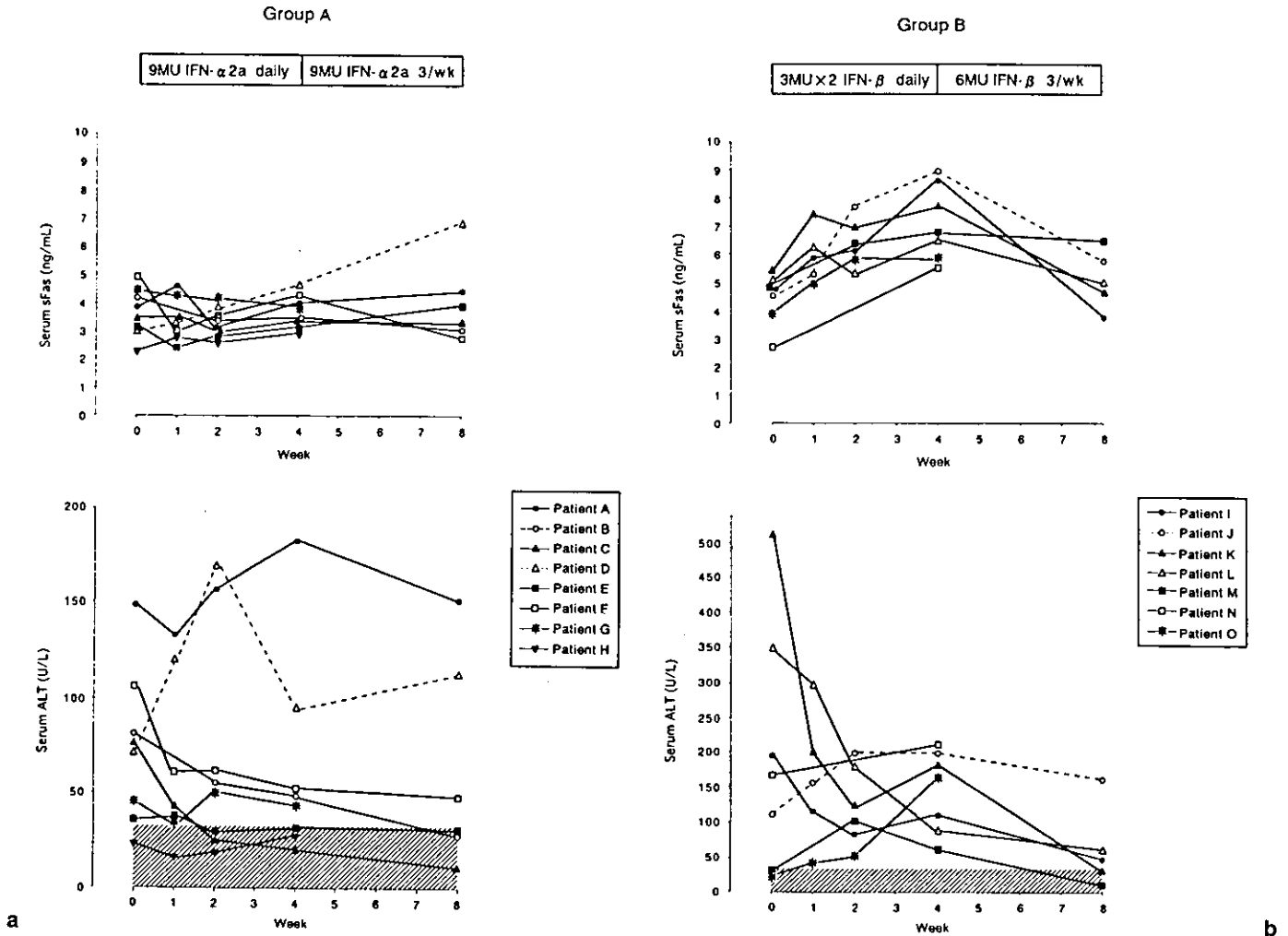
weeks). In parallel with the elevation of sFas levels, serum ALT activity was also elevated in the absence of HCV viremia in 5 of the 6 virological responders.

A sustained virological response, defined as serum HCV RNA clearance at 6 months posttreatment, was achieved by five patients (four patients in group A and one patient in group B). Table 1 shows the clinical and virological characteristics of the sustained virological responders and those of the patients showing no sustained virological response. A significant difference was observed only for the pretreatment viral load. All sustained virological responders had a low pretreatment viral load of less than 0.5 Meq/ml, whereas the levels ranged from less than 0.5 to 20.0 Meq/ml (median, 7.3 Meq/ml) in patients with no sustained virological response ( $P = 0.014$ ). Pretreatment serum sFas levels were the same for patients with a sustained virological response and those without ( $4.5 \pm 0.6$  vs  $3.8 \pm 1.0$  ng/ml;  $P = 0.089$ ). Multivariate analysis also showed only the pretreatment viral load to be predictive of a sustained virological response ( $P = 0.038$ ).

## Discussion

Currently available information suggests that apoptosis-inducing death ligands and corresponding receptors mediate cell death in human liver diseases. Thus, knowledge regarding how this system functions may help in developing rational therapeutic strategies to ameliorate clinical liver diseases. Apoptosis-inducing death receptors belong to the tumor necrosis factor/nerve growth factor receptor superfamily. Fas-Receptor (APO-1/CD95), one of the death receptors, has a single membrane-spanning domain and is expressed in hepatocytes. The particular importance of the Fas-Receptor in chronic hepatitis C has been amply documented.<sup>1,2</sup> Hepatic Fas-Receptor expression has been shown to be upregulated in HCV infection, with a close correlation to the necroinflammatory grade. The Fas-Receptor-mediated apoptotic pathway may also play a significant role in the pathogenesis of other forms of liver injury. An alternatively spliced soluble form of the Fas-Receptor without the transmembrane-spanning domain is produced by Fas-expressing hepatocytes and is considered to serve to bind Fas ligand on cytotoxic T cells, thereby minimizing liver injury.<sup>7-9</sup> However, its clinical relevance has not been well explored.

The present study demonstrated that patients with chronic HCV infection had higher levels of circulating sFas than healthy individuals without previous HCV exposure and patients who had a remote history of HCV infection but had been cleared of HCV spontaneously or following IFN therapy. sFas levels in chronic HCV infection had no relation to HCV replication



**Fig. 5a,b.** Changes in serum sFas and ALT levels during 8 weeks of enhanced IFN therapy for chronic hepatitis C. **a** Eight patients (patients A-H) received 9 megaunits (MU) of IFN- $\alpha$ 2a daily for 4 weeks, followed by the same dose thrice a week for the subsequent 4 weeks (*group A*). **b** The other patients (patients I-O) received 3MU of IFN- $\beta$  twice a day continuously for 4 weeks, and then 6MU of IFN- $\beta$  thrice a week for 4 weeks (*group B*). The *Solid* and *dashed lines* in **a** and **b** indicate patients who were negative and positive for serum HCV RNA, respectively, at the end of treatment. In the end-of-treatment virological responders, serum sFas levels showed no significant change during IFN- $\alpha$ 2a therapy in group A, while the levels increased following the initial 4-week intensive IFN- $\beta$  therapy in group B ( $4.5 \pm 0.9$  before treatment vs  $7.1 \pm 1.3$  ng/ml at 4 weeks;  $P = 0.018$ ) and returned to the baseline levels at 8 weeks. Serum sFas elevation at 4 weeks was associated with serum ALT elevation observed during IFN- $\beta$  therapy. *Shaded areas* indicate the normal range of serum ALT activity

and genotypes, but were correlated with biochemical and histological disease activity. Serum sFas levels measured by the assay we used have been shown to correlate with hepatic Fas expression.<sup>10</sup> Hepatic Fas expression in chronic hepatitis C is particularly prominent at the advancing edge of piecemeal necrosis.<sup>1</sup> sFas levels were also correlated with the severity of necro-inflammatory reactions, especially that of piecemeal necrosis. Collectively, the data obtained suggest the upregulation of hepatic Fas expression and the involvement of the Fas-mediated pathway in HCV-related liver injury. In contrast with experimental studies, sFas in our study failed to protect hepatocytes against Fas-

mediated liver injury. This discrepancy may be attributable to the amount of circulating sFas protein. The inhibition of Fas-Receptor-mediated liver injury by sFas has been reported in mice which were given very large amounts of sFas.<sup>8</sup> Serum sFas levels in mice could have been much higher than those observed in humans, and the levels in chronic hepatitis C patients may not be enough to inhibit the apoptosis caused by Fas ligand on cytotoxic T lymphocytes and its soluble form. Further studies are necessary to clarify the impact of sFas on the Fas-mediated pathway in humans. Thus far, elevation of sFas levels in chronic HCV infection has also been reported,<sup>10</sup> but controversy remains.<sup>11</sup> The



**Table 1.** Factors predictive of a sustained virological response to IFN therapy with 4-week enhanced induction treatment for chronic hepatitis C

Characteristics	Sustained response (n = 5)	No sustained response (n = 10)	P
Age (years)	50 ± 12 52 (37–63)	47 ± 9 45 (33–58)	0.540
Sex (M/F)	4/1	6/4	0.600
Serum sFas (ng/ml)	4.5 ± 0.6 4.4 (3.7–5.3)	3.8 ± 1.0 3.7 (2.3–5.1)	0.178
HCV genotype (1/2)	1/4	8/2	0.089
Serum HCV RNA titer (Meq/ml)	<0.5 (<0.5)	7.3 (<0.5 to 20.0)	0.014
Serum ALT (U/l)	177 ± 189 105 (43–508)	108 ± 103 75 (21–345)	0.327
Histological grading score	7.4 ± 2.3 8 (5–10)	5.0 ± 1.5 5 (3–7)	0.072
Histological staging score	2.2 ± 1.1 3 (1–3)	1.7 ± 1.3 1 (0–3)	0.463
Total IFN dose (MU)	863 ± 302 891 (384–1206)	611 ± 271 597 (276–990)	0.111

Values are given as means ± SD and medians (ranges)

different findings may be attributable to differences in the necroinflammatory grade of the study populations and the different sFas assays used.

In the present study, changes in sFas levels following type 1 IFN administration were investigated. IFN- $\alpha$  and IFN- $\beta$ , members of the type 1 IFN family, are currently used to treat a number of infectious diseases and cancers. A growing number of chronic hepatitis C patients have been treated with type 1 IFNs, but the effectiveness of these agents is often limited due to adverse side effects. The pathophysiological mechanisms have not been well defined. Type 1 IFN-induced hepatotoxicity is one of such adverse effects and causes serum ALT elevation in patients with a virological response. Recently, several studies showed that the antiviral effects were more pronounced in patients treated twice daily with IFN- $\beta$  compared with once-daily dosing, but that IFN-induced liver injury was more common with the twice-daily treatment protocol.<sup>15,16</sup> The present study showed that virological response to high-dose type 1 IFN therapy was not accompanied by a reduction of sFas levels and that serum ALT levels were likely to remain elevated, despite the clearance of circulating HCV RNA. Interestingly, sFas levels in virological responders increased during intravenous twice-daily treatment with IFN- $\beta$ , and the increase was accompanied by an exacerbation of biochemical disease activity.

Acute hepatopathy with elevated serum aminotransferase levels has also been reported in sheep treated with twice-daily administration of IFN tau, a type 1 IFN, and apoptosis was shown to play a critical role in the IFN tau-induced hepatotoxicity.<sup>4</sup> Type 1 IFNs can affect the expression of various genes and induce apoptosis, but the mechanism of the type 1 IFN-induced apoptosis

remains to be explored. Thus far, regulatable expression of the IFN-induced double-stranded RNA-dependent protein kinase, PKR, has been shown to trigger apoptosis, possibly through upregulation of the Fas-Receptor.<sup>17,18</sup> The data obtained in our study support this hypothesis. Elevation of sFas levels and serum ALT activity in virological responders to IFN indicates IFN-induced upregulation of hepatic Fas-Receptor expression and its involvement in type 1 IFN-induced hepatotoxicity. Again, we found that sFas exerted no apparent inhibition of Fas-mediated liver injury during IFN therapy, thus raising further questions as to whether the Fas-mediated pathway is controlled by sFas in humans. Recently, it has been reported that upregulation of sFas can be induced in the very early phase of IFN therapy.<sup>11</sup> Further studies are necessary to work out sFas dynamics in more detail during various treatment regimens.

At present, it remains to be worked out whether sFas levels in chronic hepatitis C are relevant to the therapeutic efficacy of IFN. In the present study, the majority of the patients achieved an end-of-treatment virological response after the enhanced IFN treatment, irrespective of the pretreatment patient characteristics. However, a sustained virological response was related to only the pretreatment viral load. Thus, it was shown that pretreatment circulating sFas levels have little impact on the final therapeutic outcome when patients are subjected to enhanced IFN therapy. Finally, it is likely that upregulation of the Fas-mediated pathway also occurs in other types of liver injury and is accompanied by changes in serum sFas levels. More studies are necessary to address this issue and to further our understanding of sFas.

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## C型慢性肝炎に対するインターフェロン $\alpha$ -2bとリバビリン 併用療法におけるヘモグロビン減少に関する検討

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### Study on Decreased Hemoglobin Levels in Combination Therapy with Interferon $\alpha$ -2b and Ribavirin for Chronic Hepatitis C

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The approval of ribavirin for reimbursement under the health insurance scheme in Japan in 2001 started a new era in interferon (IFN) therapy against chronic hepatitis C, enabling a change from IFN mono therapy to combination therapy with IFN and ribavirin. As the major safety problems of ribavirin, teratogenicity, hemolytic anemia and skin symptoms may be given. Also, there is known to be a greater incidence of decreased hemoglobin (Hb) in combination therapy with IFN $\alpha$ -2b and ribavirin than in the case of IFN mono therapy. Despite this, however, it has been considered that combination therapy may be accomplished if the criteria for reducing the dosage of ribavirin are strictly followed.

In this study, patients receiving combination therapy with IFN $\alpha$ -2b and ribavirin in Osaka National Hospital were followed up to evaluate the decrease in Hb in cases in which the ribavirin was reduced in dosage or discontinued (reduced/discontinued group). Hb levels in this group decreased by  $3.0 \pm 1.6$  g/dL on average after 4 weeks, significantly lower than pretreatment levels. In these subjects, it seemed that the decreased Hb levels after 4 weeks of the combination therapy aggravated anemia symptoms and subjective symptoms such as general fatigue, with the result that combination therapy could not be accomplished.

**Key words** — chronic hepatitis C, interferon therapy, ribavirin, decreased hemoglobin level

### 緒 言

C型慢性肝炎に対するインターフェロン(IFN)療法が、1992年に導入されてから10年以上が経過した。日本人の場合、C型肝炎ウイルス(HCV)の遺伝子型が genotype1bでHCV-RNA量が分岐鎖DNAプローブ法で1 Meq/mL以上、またはアンプリコア定量法で100KIU/mL以上あるIFN難治性症例が多く、これまで林ら<sup>1)</sup>や加藤ら<sup>2)</sup>、奥新らなど<sup>3,4)</sup>によりIFN療法における投与方法の工夫が行われてきた。1998年にMcHutchisonら<sup>5)</sup>やPoynardらなど<sup>6,7)</sup>が、C型慢性肝炎患者に対してリバビリン

とIFN $\alpha$ -2bの併用療法とIFN $\alpha$ -2b単独療法での臨床比較試験を行い、併用療法の高い治療効果と安全性について報告した。現在、欧州肝臓学会(EASL)やアジア太平洋肝臓学会(APASL)などにおいて併用療法を標準的治療法としている。わが国でも1998年から2000年にかけて行われた臨床比較試験の結果<sup>8,9)</sup>、2001年にリバビリンが保険適応になり、C型慢性肝炎に対するIFN療法は、IFN単独療法からIFNとリバビリンの併用療法という新しい時代になった。リバビリンの安全性上の問題点として、催奇形性と溶血性貧血、皮膚症状などが挙げられる。

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療センター)で行われた併用療法施行症例において追跡調査を行い、リバビリンの減量、または併用療法中止症例(減量・中止群)における要因を明らかにすることを目的に、ヘモグロビン(Hb)値の推移と減少量などについての検討を行ったので報告する。

## 対 象

平成14年2月より平成15年3月までに大阪医療センター消化器科において、C型慢性肝炎患者で高ウイルス量(HCV-RNA量が分岐鎖DNAプローブ法で1 Meq/mL以上)のIFN初回治療症例、またはIFN治療後再燃症例に対してリバビリンとIFN $\alpha$ -2bの併用療法を行った全症例を対象とした。投与方法は、IFN $\alpha$ -2b 6~10MIU/dayを2週間連日投与後、週3回2週間間歇投与に、リバビリン600mg(体重60kg未満)~800mg(体重60kg以上)/dayを連日併用投与とした。

## 方 法

併用療法施行患者を6カ月間の継続終了群と減量・中止群の2群に分類した。両群において、投与開始2週後、4週後のHb値の推移について投与前値との比較を行った。両群間では投与開始4週後のHb値、およびHb減少量について比較検討を行った。投与前Hb値による(投与前Hb値<12g/dL, 12g/dL $\leq$ Hb値<14g/dL, 14g/dL $\leq$ Hb値の3群に分類)リバビリンの減量、併用療法中止の割合、投与開始4週後のHb減少量による(Hb減

少量 $\geq$ 3.0g/dL, Hb減少量<3.0g/dLの2群に分類)リバビリンの減量、併用療法中止の割合について調査した。合併症および既往歴については、診断記録より調査した。リバビリンの減量および投与中止基準として、Hb値10g/dL未満で200mg減量とし、8.5g/dL未満でIFNとともに投与中止とした。Hb減少以外の有害事象が発現し、投与量の減量または併用療法中止を行う必要がある場合は、主治医の判断でそれを行うこととした。Hb値の推移における投与前値との比較および群間比較には分散分析を使用し、Hb減少量の群間比較には対応のないt検定を使用した。有意差の判定基準は $p<0.05$ を有意差とした。

## 結 果

併用療法施行症例は44例であった。うち1例は、抗ウイルス効果が認められないため、主治医の判断により併用療法が中止となったため除外した。解析対象症例43例のうち、IFN初回治療症例が6例、再燃症例が37例であり、継続終了群は23例、減量・中止群は20例(リバビリンの減量症例は7例、併用療法の中止症例は13例)であった。減量・中止群における平均年齢は、62.1 $\pm$ 8.4歳と継続終了群よりも有意に高く、併用療法開始前の血小板数(PLt)は、平均11.6 $\pm$ 3.2 $\times 10^4/\mu$ Lと有意に低値であった。患者背景をTable 1に示す。リバビリンの減量理由の内訳は、Hb値が減量基準に達した症例が6例、併用療法による全身倦怠感症状のため主治医が判断した症例が1例であった。併用療法中止理由の内訳は、Hb値が

Table 1. 患者背景

背景因子	継続終了群	減量・中止群	検定
症例(M/F)	23(18/5)	20(11/9)	
年齢(歳)	56.3 $\pm$ 10.9	62.1 $\pm$ 8.4	$p<0.05$
セロタイプ(1/2)	19/2 不明 2	16/4	
IFN投与量(6MIU/10MIU)	16/7	11/9	
Rib投与量/day(600mg/800mg)	12/11	11/9	
体重当たりのRib投与量(mg/kg/day)	11.3 $\pm$ 2.4	11.8 $\pm$ 1.1	( $n=21$ ) (n=14) N.S
AST(IU/L)	79.6 $\pm$ 39.8	75.0 $\pm$ 26.5	N.S
ALT(IU/L)	102.2 $\pm$ 77.2	95.0 $\pm$ 37.7	N.S
T-bil値(mg/dL)	0.9 $\pm$ 0.3	0.8 $\pm$ 0.3	N.S
RBC( $\times 10^4/\mu$ L)	4.4 $\pm$ 0.5	4.3 $\pm$ 0.6	N.S
WBC( $\times 10^3/\mu$ L)	4.9 $\pm$ 1.6	4.3 $\pm$ 1.1	N.S
PLt( $\times 10^4/\mu$ L)	15.7 $\pm$ 6.4	11.6 $\pm$ 3.2	$p<0.05$
Hb値(g/dL)	13.9 $\pm$ 1.8	13.7 $\pm$ 1.5	N.S
Scr(mg/dL)	0.8 $\pm$ 0.2	0.7 $\pm$ 0.1	$p<0.05$
BUN(mg/dL)	15.5 $\pm$ 4.5	15.6 $\pm$ 2.7	( $n=19$ ) N.S

mean $\pm$ S. D  
対応のないt検定  
 $P<0.05$  N. S. :not significant