patocarcinogenesis still occurs in patients with SVR (17-31). In the studies of Toyoda et al (17) and Ikeda et al (18), the risk factors for carcinogenesis were not discussed due to few sustained virological responders with carcinogenesis. Tokita et al (19) and Kobayashi et al (20) indicated that the risk factors of hepatocarcinogenesis after elimination of HCV RNA are severe fibrosis, male sex, and regular consumption of moderate amounts of alcohol, and old age at the start of IFN treatment. Their hazard ratios could not be estimated because of the relatively small number of patients with SVR. Ikeda et al (21) indicated the hazard ratios of risk factors; older age, increased aspartate aminotransferase (AST), and decreased platelet count. However, the study population was restricted to patients who received IFN monotherapy and it did not include patients who received either pegylated interferon (PEG IFN) or combination therapy of IFN and ribavirin.

The aims of this study were to estimate the rate of hepatocellular carcinogenesis in patients with chronic HCV infection who show SVR to IFN monotherapy or combination therapy of IFN and ribavirin and to determine the risk factors that affect carcinogenesis rate in such patients using multivariate analysis.

Patients and Methods

Study population

In this retrospective cohort study, all patients with chronic HCV infection who started IFN therapy between February 1987 and July 2006 in the Department of Hepatology, Toranomon Hospital were analyzed in the database. Prior to IFN therapy, they were positive for anti-HCV (second- or third generation anti-HCV, enzyme-linked immunosorbent assay, Dainabot, Osaka, Japan) and HCV RNA. Anti-HCV was assayed using stored frozen sera at -80°C. HCV RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc., Belleville, NJ) or the branched DNA probe assay (b DNA probe assay; version 2.0; Chiron, Dai-ichi Kagaku, Tokyo, Japan). The medical records of 1,193 patients with HCV infection, who had achieved HCV RNA elimination after IFN therapy or the combination therapy of IFN and ribavirin were obtained. The sera of all patients were negative for hepatitis B surface antigen (HBsAg; radioimmunoassay, Austria, Abbott Laboratories, Detroit, MI). The study protocol was approved by the Human Ethics Review Committee of Toranomon hospital.

Clinical background and laboratory data

The background of 1,193 patients who achieved SVR is shown in Table 1. They included 809 men and 384 women, who were 15 to 83 years old with a median age of 50 years at the commencement of therapy. HCV genotype was analyzed by the immunoserological typing method using a commercial kit (Kokusai Diagnostic Corporation, Kobe, Japan).

Table 1. Patients' Profiles, Virological, Histological Characteristics of the Patients Prior to Their Interferon (IFN) Therapy and Protocol of IFN Therapy

Number of patients	1193
Sex (M / F)	809 / 384
Age (years) *	50 (15-83)
Observation period (year) *	8.3 (0.0-19.0)
HCV genotype	
Genotype 1a, 1b	494 (41.4%)
Genotype 2a, 2b	670 (56.2%)
Genotype 1+2	5 (0.4%)
Genotype 3	1 (0.1%)
Undetermined	23 (1.9%)
Histological stage of hepatitis	
F0 (no fibrosis)	7 (0.6%)
F1 (slight fibrosis)	738 (61.9%)
F2 (moderate fibrosis)	289(24.2%)
F3 (severe fibrosis)	72 (6.0%)
F4 (cirrhosis)	41 (3.4%)
Not examined	46 (3.9%)
IFN therapy	
Monotherapy	1032 (86.5%)
Combination therapy with ribavirin	161 (13.5%)
Type of IFN	
IFN-α	850 (71.2%)
PEG IFN-α	46 (3.9%)
IFN-β	251 (21.0%)
IFN-α/PEG IFN-α+IFN-β	47 (3.9%)

IFN: interferon, PEG IFN: pegylated interferon

*Data are median (minimum, maximum) values.

The HCV genotype was 1 (genotype 1a and 1b) in 494 patients, 2 (genotype 2a and 2b) in 670 patients, 1 plus 2 in 5 patients, 3 in 1 patient. Before treatment, 1,131 patients underwent liver biopsy with or without peritoneoscopy to assess the staging of liver fibrosis and the grade of inflammatory activity based on the classification of Desmet (32). Staging of liver fibrosis was defined as F0 (no fibrosis), F1 (fibrosis portal expansion), F2 (bridging fibrosis), F3 (bridging fibrosis with architectural distortion) and F4 (cirrhosis). Additionally, 16 patients were diagnosed as cirrhosis by peritoneoscopy without biopsy, laboratory values or clinical features: 1147 patients were diagnosed with chronic hepatitis (n=1,106) and cirrhosis (n=41) (F0/F1/F2/F3/F4=7/738/289/72/41).

Treatment protocol

IFN was performed once in 973 patients and more times of therapy in 220 patients (twice/three times/four times/five times/six times=166/38/13/2/1). IFN and ribavirin combination therapy was used to eliminate HCV RNA for 161 patients, while IFN monotherapy eliminated HCV RNA for the other 1,032 patients. The type of IFN was IFN-α (natural or recombinant)/PEG IFN-α in 896 patients (75.1%); IFN-α in 850 patients (71.2%), PEG IFN-α in 46 patients (3.9%), IFN-β (natural) in 251 patients (21.0%) and IFN-α or PEG IFN-α and IFN-β in 47 patients (3.9%).

A total of 613 patients (51.4%) received 3 to 9 million units of IFN everyday for 8 weeks followed by twice or three times a week for 1 to 305 weeks (for 16 to 22 weeks in 75% of patients), 304 patients (25.5%) received 3 to 9 million units of IFN everyday for 1-5 weeks followed by three times a week, 5 patients (0.4%) for 12 weeks and one patient (0.1%) for 24 weeks followed by intermittent administration. A total of 124 patients (10.4%) underwent short therapy with IFN everyday for 4-8 weeks, 2 patients (0.2%) for 10-12 weeks, 18 patients (1.5%) for 18-24 weeks. 2 patients (0.2%) had a prolonged administration of IFN for 11 and 13 months. And 63 patients (5.3%) underwent intermittent administration of three times a week for 4weeks to 70 months. This protocol is one of the low-dose intermittent IFN therapies. A total of 48 patients (4.0%) underwent 50-180 μg of PEG IFN once a week: 8 patients for 24 weeks and 40 patients for 48 weeks.

Follow-up and diagnosis of hepatocellular carcinoma

Almost all patients were followed-up every week or biweekly during IFN monotherapy. This included hematological, biochemical, and virological tests. Patients treated with pegylated IFN were also checked every week or biweekly. After the completion of treatment, monthly follow-up was continued until the virological response could be determined. When SVR was confirmed, imaging studies were conducted once or twice per year in the majority of patients; these included computed tomography (CT) or ultrasonography (US), except those patients who were lost to follow-up. Angiography was performed only when HCC was highly suspected on CT or US. The presence of a characteristic hypervascular nodule on angiography was considered a specific finding for HCC, and histological confirmation was usually not required in the majority of such cases. The clinical trends of tumor markers were also taken into account. When angiography could not be performed, the hepatic mass was considered HCC when CT showed a hypervascular mass and the tumor marker level was elevated. No fine needle biopsy or histopathological examination was performed before treatment.

The date of the last follow-up in this study was March 1, 2007. The median observation period of the entire group was 8.3 years with a range of 0.0 to 19.0 years. As for patients of the combination therapy, the median follow-up period was 3.2 years with a range of 0.0 to 7.5 years.

Statistical analysis

Non-parametric procedures were employed for the analysis of background clinicopathological parameters, including Mann-Whitney U-test. The rate of hepatocarcinogenesis was calculated for the period between the end of IFN therapy and appearance of HCC, using Kaplan-Meier technique (33). Differences in carcinogenesis curves were tested using the log-rank test. Independent factors associated with the appearance of HCC were studied using stepwise Cox regression analysis (34). The following seven variables were analyzed for potential covariates for liver carcinogenesis; age, sex, fibrotic stage of hepatitis at the initiation of the IFN therapy, HCV genotype, use of ribavirin (monotherapy or combination therapy), type of IFN (α or β, and number of treatments. Factors found significant were entered into a multivariate Cox proportional hazard model. A P-value less than 0.05 was considered significant. Data were analyzed using the SPSS software ver. 11.0.1J (SPSS Inc., Chicago, IL).

Crude rates of hepatocarcinogenesis

During a median observation period of 8.3 years with a range of 0.0 to 19.0 years, HCC was diagnosed in 23 (1.9%) of the 1,193 patients. The median interval between the end of therapy and detection of HCC was 3.1 years (range, 0-12.9 years).

The characteristics of HCC patients are shown in Table 2. Patients who developed HCC before the initiation of IFN therapy were excluded. Four patients (Nos. 2, 5, 15 and 22) developed HCC before the diagnosis of SVR but after the elimination of HCV RNA. The surgically resected liver tissue was also examined by the PCR method in 4 cases (Nos. 1, 6, 13 and 14), which showed no HCV RNA.

HCC patients included 21 men and 2 women; the median age at the start of IFN therapy and at diagnosis of HCC was 58 (range, 50-70) and 62 (51-76), respectively. The HCV genotype was 1 in 9 patients and 2 in 14 patients. Chronic hepatitis was diagnosed in 14 patients (F1/F2/F3=2/12/0) and cirrhosis in 9, at the time of initiation of IFN therapy. The type of therapy for hepatitis was IFN monotherapy in 22 patients and the combination therapy in 1. The number of HCC tumors was one in 18 patients, two in 3 patients and more than two in 2 patients. A typical hypervascular mass on angiography or perfusion defect on CT during arterial portography (CT-AP) was noted in 20 patients. Angiography could not be performed in the other three patients; they had a hypo-enhanced, iso enhanced, and hyper-enhanced tumor on CT, respectively. Treatment was radical in 21 patients; including hepatectomy in 17 and percutaneous locoregional therapy in 4 patients. At the time of surgical resection, the fibrosis staging was histopathologically examined in 16 patients. Twelve patients was diagnosed as hepatitis (F1/F1-2/F2/F3=2/1/6/3) and 4 as cirrhosis. In

Table 2. Carcinogenesis after HCV RNA Elimination

No	Gender	Age at the start of IFN	Age at the carcinogenesis	Genotype	Type of IFN	Fibrosis staging before IFN Tx	Interval between the end of IFN Tx and carcinogenesis, yr	Number of Tumor	Tumor size, mm	Treatment for HCC	Fibrosis staging at the time of carcinogenesis	Differentiation of HCC
1	M	50	51	1b	α	F2	1.0	1	15	Hepatectomy	F3	Moderate
2	M	52	54	2a	α	F1	0.6	1	18	Hepatectomy	F2	Well
3	F	54	59	2a	α	F2	3.5	1	17	Hepatectomy	F1-2	Moderate
4	M	55	60	1b	α	F2	3.7	1	16	Hepatectomy	F2	Moderate
5	M	55	56	1b	Peg α+Rib	F2	0.0	1	21	RFA	-	-
6	M	55	57	2a	α2a	F4	1.9	1	19	Hepatectomy	F4	Moderate
7	M	57	67	2	α	F2	8.9	1	47	Hepatectomy	F1	Moderate
8	M	55	59	2a	α2a	F4	3.1	1	18	Hepatectomy	F4	Moderate
9	M	55	62	1b	β	F4	6.5	1	16	Hepatectomy	F4	Poor
10	M	57	58	1b	α	F2	0.9	1	16	Hepatectomy	F2	Moderate
11	M	57	59	1b	α	F2	1.2	1	20	Hepatectomy	F2	Moderate
12	M	58	66	2a	α2b	F1	8.7	1	26	Hepatectomy	F1	Well
13	M	58	62	1b	β	F2	3.9	1	30	Hepatectomy	F2	poor>moderate
14	M	59	69	2b	α	F2	9.1	1	21	Hepatectomy	F2	Moderate
15	M	59	61	1b	α	F4	0.1	1	30	Hepatectomy	F3	poor>moderate
16	M	61	63	2	α	F2	1.8	4+LN meta	23	Hepatectomy+MCT	F3	moderate>well
17	M	62	65	2a	β	F4	2.4	2	20,20	RFA	-	-
18	M	62	75	2a	α2a	F4	12.9	1	23	Hepatectomy	F4	Moderate
19	M	63	66	2a	β	F2	3.6	Uncountable	Diffuse	No treatment	-	
20	M	65	71	2a	α	F2	5.0	2	12, 8	Hepatectomy	-	Necrosis
21	F	66	68	2a	α	F4	0.9	1	13	RFA	-	-
22	M	69	72	1b	β	F4	0.4	2	13, 13	Hepatectomy	-	moderate, poor
23	M	70	76	2a	β	F4	5.4	1	10	RFA+PMCT	-	-

IFN: interferon, Tx: therapy, Peg: pegylated interferon, Rib: ribavirin, LN meta: lymph node metastasis,
RFA: radiofrequency ablation, MCT: microwave coagulation therapy, PMCT: percutaneous microwave coagulation therapy.

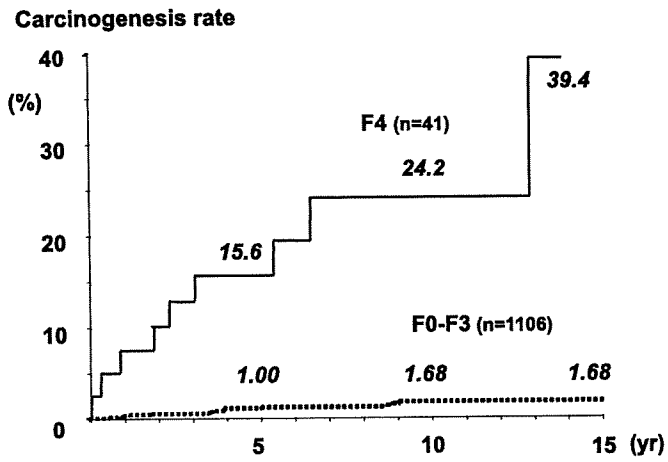

Figure 1. Rates of hepatocarcinogenesis in 41 patients with cirrhosis (F4) and 1,106 patients with liver fibrosis stage F0-F3.

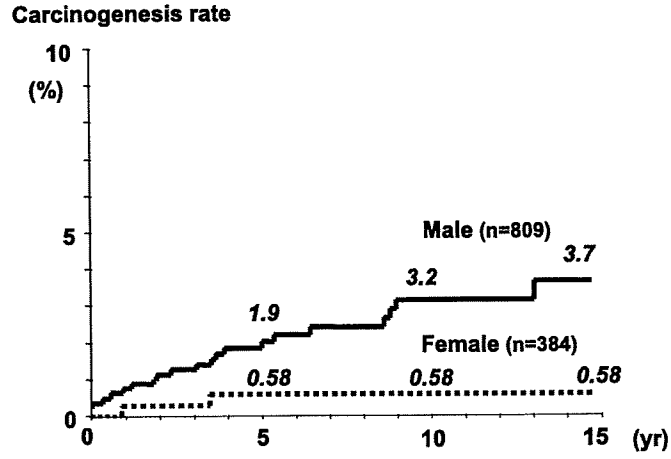

Figure 2. Rates of hepatocarcinogenesis in 809 male patients and 384 female patients.

comparison with the staging at the initiation of IFN therapy, 3 cases showed improvement in the fibrosis, 10 showed no change, and 3 showed progression.

The crude rates of hepatocarcinogenesis in the SVR patients were 1.5%, 2.4% and 2.7% at the end of the 5th year, 10th year and 15th year, respectively.

Determinants of hepatocarcinogenesis

The rate of carcinogenesis was significantly higher in 41 patients with cirrhosis (F4) than in 1,106 patients with liver fibrosis of F0-F3 ($p<0.0001$, Fig. 1). The respective cumulative HCC development rates in patients with cirrhosis at 5, 10, and 15 years after SVR were 15.6%, 24.2% and 39.4%. On the other hand, the respective rates for patients with F0-F3 were 1.00%, 1.68% and 1.68% at 5, 10, and 15 years after SVR. When patients were divided into two groups with F2-4 and with F0-1, rates of the former group were 4.16%, 6.52% and 7.58%, while those of latter group were 0.13%, 0.40% and 0.40%. The incidence rates of HCC increases with the fibrotic stage; those with F1, F2 and F4 were 0.14%, 3.43% and 15.6% at 5 years, 0.40%, 5.22% and 24.2% at 10 years, and 0.40%, 5.22% and 39.4% at 15 years, respectively.

The rate of hepatocarcinogenesis among 809 male patients was significantly higher than among 384 female patients ($p=0.018$, Fig. 2); the respective rates at 5, 10 and 15 years were 1.87%, 3.18% and 3.67% for males and 0.58%, 0.58% and 0.58% for females.

The rate of hepatocarcinogenesis among 570 patients aged >50 years was greater than among 623 patients aged <51 years at the start of IFN therapy ($p<0.0001$, Fig. 3); the respective rates at 5, 10 and 15 years for the former group were 2.92%, 4.93% and 5.81%, compared with 0.16%, 0.16% and 0.16% for the later.

Multivariate analysis identified three factors to be associated with the rate of development of HCC: sex, age at start IFN treatment, and fibrotic stage in the liver tissue. Multi-

variate analysis was performed using non-time dependent proportional hazard analysis. Fibrotic stage, sex, and age were identified as significant independent factors that influenced the rate of future hepatocarcinogenesis (Table 3). Cirrhosis (F4) was associated with a higher risk of hepatocarcinogenesis with a hazard ratio of 12.9 (95% confidence interval, 5.5-30.6, p<0.001) compared with F1-3 stage. Similarly, male sex (6.45, p=0.012) and older age than 50 years (20.2, p=0.004) were associated with a higher risk. Serological grouping of HCV, type of therapy (monotherapy or combination therapy), type of IFN of the final therapy, and number of therapies did not significantly influence the rate of hepatocarcinogenesis. When the patients were divided into two groups with F0-1 and with F2-4, hazard ratios of F2-4, male and older age were 13.4 (3.1-57.8, p<0.0001), 7.00 (1.63-29.99, p=0.0009) and 17.6 (2.3-131.6, p=0.005). When the patients were divided into two groups with F0-2 and F3-4, hazard ratios of F3-4, male and older age were 5.8 (2.5-13.8, p<0.0001), 6.78 (1.58-29.07, p=0.01) and 22.9 (3.0-172.2, p=0.002).

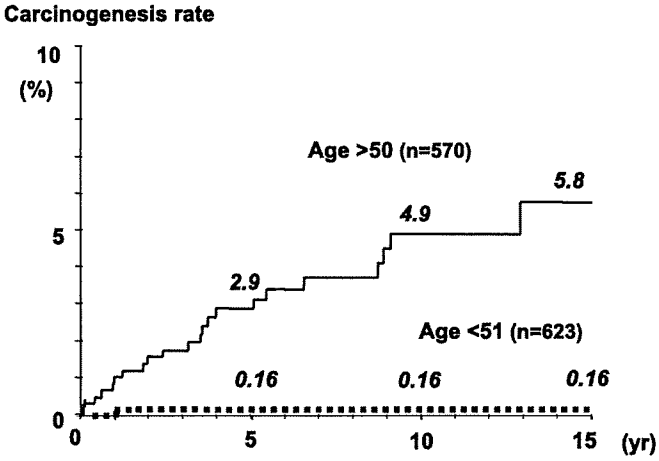

Figure 3. Rates of hepatocarcinogenesis in 570 patients older than 50 and 623 patients younger than 51 years.

Discussion

Epidemiological data on the rate of development of HCC in patients with chronic hepatitis (35) and those with cirrhosis (36) indicate that the life expectancy of patients with HCV-related chronic liver disease is significantly influenced by the development of HCC. Up to 75% of patients with HCV infection and cirrhosis eventually develop HCC (15). IFN can be considered to have anti-carcinogenic properties through its anti-inflammatory action, since several studies have already described that the cancer suppressive activity of IFN in those patients who show HCV RNA eradication was similar to that of patients with ALT normalization without HCV RNA elimination (BR) (15, 37-40). After excluding patients with cirrhosis, the previous report (40) showed that the rate of carcinogenesis was lower in patients with SVR than in those with BR because HCV-elimination does not result in re-elevation and exacerbation of ALT. As a follow-up to the above studies, the rate of hepatocarcinogenesis in SVR patients with either chronic hepatitis or cirrhosis was estimated in the present study.

In spite of the anti-carcinogenic effect of SVR, 23 cases developed HCC following elimination of HCV RNA among 1,193 patients. The median interval between the end of IFN therapy and carcinogenesis is 3.1 years with a range of 0.0 to 12.9 years. Among 23 cases, 22 patients had regular examinations of at least once a year, and 21 of them received radical treatment such as hepatectomy or radiofrequency ablation. The high rate of radical treatment was probably due to the preserved liver function after HCV RNA elimination.

HCCs in six cases that were detected in the year after the end of the interferon therapy could have been already present before elimination of HCV RNA. Even when we exclude these cases, multivariate analysis identified the same factors such as higher histological stage, male sex and age older age as determinants of hepatocarcinogenesis. The haz-

Table 3. Factors Associated with Hepatocarcinogenesis in Sustained Virological Responders with Chronic HCV Infection

Factors	Category	Hazard ratio	95% confidence interval	p
Fibrotic stage	1: F0-3	1		
	2:F4	12.9	(5.5-30.6)	<0.001
Gender	1: women	1		
	2: men	6.45	(1.51-27.64)	0.012
Age (years)	1: <51	1		
	2: >50	20.2	(2.7-152.9)	0.004

ard ratios of cirrhosis, male sex and age older than 50 years were 10.9 (4.0-29.8, p<0.001), 10.4 (1.4-78.2, p=0.024) and 17.0 (2.2-130.7, p=0.006), respectively.

The rates of hepatocarcinogenesis in patients with histological stage F0-F3 were 1.00%, 1.68%, 1.68% at 5, 10, and 15 years after SVR, respectively. These rates were about 20% less than the rates reported previously for patients with chronic hepatitis; 4.8%, 13.6%, and 26.0%, respectively (35). The rates of hepatocarcinogenesis in patients with cirrhosis were 15.6%, 24.2%, and 39.4% at 5, 10, and 15 years, respectively, which were about 65% less than the rates reported previously for patients with cirrhosis; 21.5%, 53.2% and 75.2%, respectively (36). These results indicate that IFN has a more marked effect in reducing the rate of hepatocarcinogenesis in patients with F0-F3 than in those with cirrhosis. Furthermore, the difference in the rate of hepatocarcinogenesis in patients who show SVR and those with chronic HCV-infected patients increases with time, since the likelihood of development of HCC before elimination of HCV RNA decreases as time passes after IFN therapy.

Although some studies have reported that elderly male patients with severe fibrotic stage could be at a high risk for hepatocarcinogenesis even when they show SVR, the hazard ratios in such patients have not been reported probably because of shorter follow-up period and the relatively small number of patients. In this study, the follow-up period was longer than that of previous studies, allowing meaningful multivariate analysis (e.g., Cox hazards model). The results of such analysis showed that the risk of carcinogenesis increases with the histologic stage of the liver, age and male sex. This finding was similar to that reported in a study of untreated patients (41, 42) or IFN-treated hepatitis patients with the histological stage of F0-F3 (15).

Treatment of patients with chronic HCV infection using PEG IFN-α and ribavirin resulted in persistently negative tests for serum HCV RNA in 40-50% of patients with HCV genotype 1 and 75-80% with HCV genotype 2 or 3. The present study also showed that neither type of IFN (α or β) nor the use of ribavirin altered the rate of carcinogenesis. Further studies are needed with a longer follow-up period since the follow-up period of patients treated with the combination therapy ranged from only 0 to 7.5 years (median 3.2years) and was shorter than that of patients who received IFN monotherapy.

In conclusion, the results emphasize the importance of long-term follow-up of patients with chronic HCV infection, even those who show SVR to IFN therapy, especially male elderly patients with severe fibrosis of the liver.

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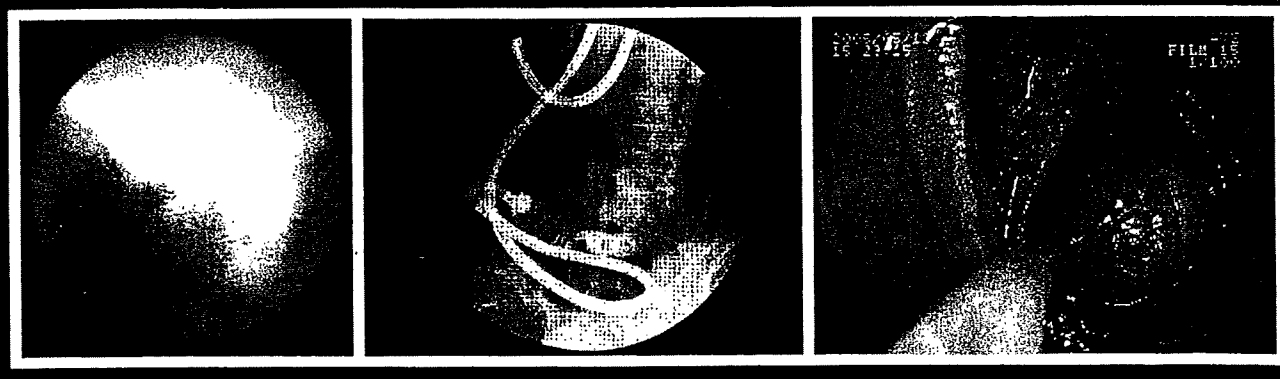

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Repeated administration of recombinant human serum albumin caused no serious allergic reactions in patients with liver cirrhosis: a multicenter clinical study

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Background. We carried out a multicenter study to evaluate the safety of recombinant human serum albumin (rHSA), developed using the methylotrophic yeast *Pichia pastoris,* during and after repeated administration in patients with liver cirrhosis. ***Methods.*** rHSA was administered to 423 cirrhosis patients with ascites or edema. rHSA was administered three times over 3 days, and each 3-day treatment course was repeated at least three times with an interval of at least 2 weeks between courses. Adverse drug reactions (ADRs) were monitored during and after repeated rHSA administration. Specific antibody titers against *Pichia* yeast components were measured before and after treatment. Efficacy was evaluated on the basis of changes in serum albumin level, colloid osmotic pressure, and body weight. ***Results.*** ADRs were observed in 96 of 423 patients (22.7%), with no serious allergy or difference in the incidence of ADRs observed among the first, second, and third administrations. Specific IgE and IgG antibodies were detected before treatment in 19 and 422 patients, respectively. However, allergic ADRs were observed in 14 patients in whom specific IgE antibodies were not detected. No obvious relationship between allergic ADRs and specific IgE or IgG titers was identified. Serum albumin levels and colloid osmotic pressure increased significantly ($P < 0.0001$), and body weight decreased significantly ($P < 0.0001$) after rHSA administration. ***Conclusions.*** rHSA caused no serious allergic reactions even when three treatment courses were administered at intervals of at least 2 weeks.

Key words: recombinant human serum albumin (rHSA), *Pichia pastoris*, liver cirrhosis, allergic reactions, antibody testing

Introduction

Plasma-derived human serum albumin (pHSA) is used to correct circulating plasma volume and improve colloid osmotic pressure. As pHSA is manufactured from human blood, the supply is limited and the risk of infection by unknown viruses or prions cannot be completely ruled out. To alleviate these problems, recombinant human serum albumin (rHSA) has been developed from *Pichia pastoris*[1] without using any animal-derived materials.

rHSA and pHSA have been shown to be identical in structure and physicochemical and immunochemical properties.[2–4] A phase III controlled clinical study of patients in liver cirrhosis, with increased serum albumin as the primary end point, showed that the efficacy of rHSA is comparable to that of pHSA.[5,6] A similar efficacy has been demonstrated in patients with hemorrhagic shock, thermal burns, or nephrotic syndrome.[5,6] No serious allergic symptoms were observed in these studies.[5,6]

At present, many recombinant pharmaceuticals with established efficacy and safety are being used, and among blood products, recombinants of blood coagulation factor VIII[7,8] are increasingly replacing plasma-derived preparations. Despite the high purity of rHSA, the potential onset of allergic reactions triggered by *Pichia* yeast components must be investigated, because the administered doses of rHSA are higher than those of other recombinants, and because administration might be repeated. This multicenter study was therefore conducted in cirrhosis patients to evaluate the safety and efficacy of rHSA during and after repeated administration of rHSA.

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Methods

Patients

A total of 423 patients with liver cirrhosis [etiology: hepatitis B virus (HBV) in 59 patients, hepatitis C virus (HCV) in 264, alcohol consumption in 47, primary biliary cirrhosis (PBC) in three, autoimmune hepatitis in three, non-B, non-C hepatitis in ten, HBV plus HCV in four, HCV plus alcohol consumption in seven, and other in 26] were enrolled in this study from July 2002 to January 2005. Written informed consent was obtained from all patients.

All patients meeting the following criteria were included in this study: serum albumin level <3.0 g/dl; presence of cirrhotic ascites or edema (legs or back); and age, 20–75 years. Beginning with the second treatment course, patients with serum albumin level <3.0 g/dl or cirrhotic ascites or edema (legs or back) were eligible.

Patients who met one or more of the following criteria were excluded from each course: prothrombin time <30% or 5 s≥ reference or control time; total bilirubin ≥5.0 mg/dl; serum creatinine ≥4.0 mg/dl; presence of hepatocellular carcinoma (HCC) with tumor embolism in the portal vein (main trunk or primary/secondary branch), main trunk of the hepatic vein, or postcaval vein; New York Heart Association class III/IV; history of shock in response to any pHSA preparation component; hepatic encephalopathy of grade II or higher severity at the time of consent; and pregnancy or lactation in women. Also excluded were any patients the investigator considered inappropriate for the study.

Protocol amendment

During the course of this study, a phase I study of rHSA was conducted in the United States in healthy volunteers with high specific IgE antibodies against *Pichia* yeast components (≥0.7 U_A/ml). In the American study, serious allergic adverse drug reactions (ADRs) were observed in two of four patients (bronchospasms in one patient and bronchospasms and generalized urticaria in one patient), leading to discontinuation of the study. Consequently, new enrollment in the present study was temporarily suspended in May 2003 while the cause was investigated. A passive cutaneous anaphylaxis (PCA) reaction test in rats showed that the products used in the American study were more antigenic than those used in the present study. Afterward, purification techniques were improved and the problem was resolved.

In November 2003, the present study was restarted with an amended protocol that included the following additional inclusion criteria: specific IgE antibody titers against *Pichia* yeast components were required to be <0.35 U_A/ml before each treatment, and hospital admission is indispensable from the time of the first administration (day 1) to the day after the final administration (day 4).

Treatment

After confirmation of a negative skin prick test against Pichia yeast components, 25 g/day of rHSA (25% rHSA, Bipha, Chitose, Japan) was intravenously administered over 2 h each day for 3 days. This course of treatment was repeated for three courses, and optionally for five courses. Each course was started after an interval of at least 2 weeks after the previous course.

Concomitant medication

During the study period, concomitant use of other investigational products was not allowed. Administration of plasma protein products, blood transfusion, reinfusion of concentrated ascites, paracentesis, and invasive treatment of complications associated with the underlying disease (e.g., endoscopic variceal ligation or sclerotherapy for esophageal varices, percutaneous ethanol injection therapy, or radiofrequency ablation for HCC) were prohibited from the day the inclusion/exclusion criteria were examined, except for specific IgE testing, until day 10. Continued use of already prescribed diuretics was allowed; however, the start of diuretics was prohibited during days 1–4. Branched-chain amino acid formulae and other pharmaceuticals could be used concomitantly.

Measurements and observation

Inclusion/exclusion criteria were examined within 3 days prior to day 1 of each treatment course, and specific IgE screening, which was added as an inclusion criterion by the protocol amendment, was performed within 14 days prior to day 1.

ADRs were monitored during the study period. Hematological tests and urinalysis were performed at baseline (within 3 days prior to day 1) and on days 4 and 10, and antibody tests were performed on day 1 (pretreatment) and days 4 and 10 in each course to evaluate safety.

Serum albumin level, colloid osmotic pressure, and body weight were measured on days 1 (pretreatment) and 4 during each course to evaluate efficacy. Serum albumin level and colloid osmotic pressure were measured at Mitsubishi Chemical Medience Corporation (Tokyo, Japan)

Table 1. Descriptions of *Pichia* yeast components

PYC-a	A centrifuged supernatant of non-rHSA-producing *Pichia* yeast culture
PYC-b	A *Pichia* yeast lysate from a non-rHSA producing *Pichia* transformant
PYC-c	A *Pichia* yeast component from a non-rHSA producing *Pichia* transformant purified via passage through a streamline column

PYC, *Pichia* yeast component

Antibody testing

Specific IgE and IgG antibody titers against three types of *Pichia* yeast components, PYC-a, -b, and -c (Table 1), were measured by fluorescent enzyme immunoassay (ImmunoCAP;[9] Phadia, Uppsala, Sweden) at Mitsubishi Chemical Medience Corporation. Intraday (ten repetitions), interday (duplicate measurements over 5 days), and interinstrument (duplicate measurements using three instruments) reproducibility was assessed, yielding a maximum coefficient of variation of 11.6% and 8.6% for specific IgE and IgG antibody titers, respectively. The quantification limit was 0.35 U_A/ml and 2 mg_A/l for specific IgE and IgG antibody titers, respectively.

Data analysis

The incidence of ADRs and abnormal laboratory changes for which a causal relationship to rHSA could not be ruled out was assessed. In addition to allergic ADRs judged by each investigator, skin symptoms, including rash, drug eruption, eczema, erythema, Henoch-Schonlein purpura, purpura, pruritus, and generalized pruritus; respiratory symptoms, including cough, wheezing, rhinorrhea, and nasal congestion; and pyrexia were selected as "allergy-related ADRs" by the sponsor (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) and analyzed.

The incidence of ADRs that occurred in patients with at least one of the three types of specific IgE antibody titers detected at one or more time points was compared with those in whom antibodies were not detected at any time points.

As the three types of specific IgG antibody titers were not normally distributed, the value of each titer was logarithm-transformed to evaluate the time course. The incidence of ADRs that occurred in patients with an increase in specific IgG antibodies of ≥50% from baseline of the first course or the respective course was compared with that in patients with no increase.

Statistics

Incidences of ADRs were compared by using Fisher's exact test. Changes in serum albumin level, colloid osmotic pressure, and body weight from day 1 (pretreatment) to day 4 were evaluated using a paired t test. Two-sided P values of <0.05 were considered to indicate statistical significance. These analyses were performed with SAS statistical analysis software version 8.2 (SAS Institute, Cary, NC, USA).

Ethics

This study was conducted in compliance with good clinical practice. The protocol was reviewed and approved by the institutional review board at each study site.

Results

Screenings were conducted in 472 patients. Twenty-two, three, and four patients were excluded because of the inclusion/exclusion criteria (except for the specific IgE criterion), deterioration of hepatic cirrhosis, and other reasons, respectively. Of the original 472 patients, 39 were specific IgE positive before treatment. Of these 39 patients with positive IgE, 19 were included in this study: 18 were included before the protocol amendment in November 2003, and one patient whose specific IgE was negative in the screening test but became positive at just prior to the first course treatment was included after the protocol amendment. The remaining 20 patients were excluded because of positive IgE results. Consequently, rHSA was administered to 423 patients (267 men and 156 women) in total. At baseline, their mean age was 63.3 ± 8.1 years. Mean serum albumin level, total bilirubin, creatinine, and prothrombin time were 2.5 ± 0.3 g/dl, 1.9 ± 1.0 mg/dl, 1.0 ± 0.5 mg/dl, and 61.3 ± 16.1%, respectively. Physical findings of ascites, imaging findings of ascites, and physical findings of edema were observed in 70.2%, 84.2%, and 75.2% of patients, respectively (Table 2).

Adverse drug reactions

rHSA was administered to 423, 314, 219, 63, and 34 patients during the first, second, third, fourth, and fifth courses, respectively.

The overall incidence of ADRs was 22.7% (96/423 patients). Common (incidence ≥1%) ADRs were pyrexia (6.9%; 29/423), hepatic encephalopathy (2.4%;

Table 2. Baseline demographics

	No. of patients or mean ± SD	%
Sex		
Male	267	63.1
Female	156	36.9
Age, years	63.3 ± 8.1	
Cause of hepatic cirrhosis		
HBV	59	13.9
HCV	264	62.4
Alcohol consumption	47	11.1
PBC	3	0.7
Autoimmune hepatitis	3	0.7
Non-B, non-C	10	2.4
HBV + HCV	4	0.9
HCV + alcohol consumption	7	1.7
Others	26	6.1
Serum albumin (g/dl)	2.5 ± 0.3	
Total bilirubin (mg/dl)	1.9 ± 1.0	
Creatinine (mg/dl)	1.0 ± 0.5	
Prothrombin time[a] (%)	61.3 ± 16.1	
Prothrombin time[b] (s)	13.6 ± 1.4	
Ascites (physical diagnosis)		
−	126	29.8
+	297	70.2
Ascites (diagnostic imaging)		
−	66	15.6
+	356	84.2
Unknown	1	0.2
Edema		
−	103	24.3
+	318	75.2
Unknown	2	0.5
Hepatocellular carcinoma		
−	276	65.2
+	147	34.8
New York Heart Association		
No heart failure	397	93.9
NYHA classification		
I	24	5.7
II	2	0.5

HBV, hepatitis B virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; NYHA, New York Heart Association
[a] $n = 418$
[b] $n = 5$

10/423), rash (2.1%; 9/423), pruritus (1.7%; 7/423), and dehydration (1.2%; 5/423) (Table 3). Uncommon but significant ADRs were hemorrhagic shock (0.2%; 1/423), hemorrhoidal hemorrhage (0.2%; 1/423), gastric variceal hemorrhage (0.2%; 1/423), and gastrointestinal hemorrhage (0.2%; 1/423) (Table 3).

Incidences of skin symptoms, respiratory symptoms, and pyrexia, defined as allergy-related ADRs by the sponsor, were 5.4% (23/423), 1.4% (6/423), and 6.9% (29/423) respectively (Table 4). Incidences of overall and allergy-related ADRs did not increase during repeated administration (Table 4).

ADRs judged to be allergic symptoms by the investigators were observed in 14 patients: rash (eight patients), drug eruption (two), Henoch-Schonlein purpura (two), purpura (one), and wheezing (one). None of these allergic ADRs were serious, and they regressed or improved with appropriate intervention (Table 5). In all these patients, specific IgE antibodies against *Pichia* yeast components were not detected before or after rHSA administration.

Common (incidence ≥1%) abnormal laboratory changes for which a causal relationship with rHSA could not be ruled out were aggravated urinary occult blood (2.9%; 12/421), aggravated urine protein (2.4%; 10/421), increased blood urea nitrogen (2.4%; 10/423), decreased hemoglobin (1.9%; 8/423), increased eosinophils (1.9%; 8/422), increased neutrophils (1.9%; 8/422), decreased red blood cells (1.7%; 7/423), decreased hematocrit (1.7%; 7/423), decreased lymphocytes (1.7%; 7/422), decreased platelets (1.4%; 6/423), increased creatinine (1.4%; 6/423), increased total bilirubin (1.2%; 5/423), and aggravated urine urobilinogen (1.4%; 6/421) (Table 6).

Specific IgE antibodies and ADRs

In 19 of 423 patients, at least one of the three types of specific IgE antibodies against *Pichia* yeast component was detected before treatment. Of these patients, antibody titers increased after treatment in four patients, but not in the remaining 15 patients. In five of the 404 patients negative for specific IgE antibodies before treatment, at least one of the specific IgE antibodies became detectable after treatment, but not in the remaining 399 patients.

The incidences of overall and allergy-related ADRs in the 24 patients with specific IgE antibodies detected before or after treatment did not differ from those in the 399 patients in whom the antibodies were not detected (Table 7).

It should be taken into account that specific IgE <0.35 was added as an inclusion criterion during the course of this study, in November 2003. Nineteen patients positive for specific IgE were included in this study. None of these patients experienced allergic ADRs. Furthermore, in the subgroup of specific IgE-negative patients, the allergic ADR incidence in patients before and after protocol amendment was 4.4% (9/206) and 2.5% (5/198), respectively, which are statistically not different. Consequently, the protocol amendment might not have affected the incidence of allergic ADRs.

Specific IgG antibodies and ADRs

Specific IgG antibodies against *Pichia* yeast components were detected in 422 of 423 patients before treatment. In 88 of 423 patients, specific IgG antibody titers increased by 50% or more after rHSA administration,

Table 3. Adverse drug reactions

System organ class	Incidence		
	≥1%	0.5% to <1%	<0.5%
Infections and infestations			Cellulitis (0.2%) Folliculitis (0.2%)
Blood and lymphatic system disorders		Anemia (0.5%)	Thrombocytopenia (0.2%) Nephrogenic anemia (0.2%)
Metabolism and nutrition disorders	Dehydration (1.2%)	Hyperammonemia (0.9%)	Hyperuricemia (0.2%)
Psychiatric disorders			Disorientation (0.2%) Delirium (0.2%)
Nervous system disorders	Hepatic encephalopathy (2.4%)	Headache (0.7%)	Subarachnoid hemorrhage (0.2%) IIIrd nerve paralysis (0.2%) Syncope (0.2%) Cerebral infarction (0.2%) Convulsion (0.2%)
Cardiac disorders			Palpitations (0.2%)
Vascular disorders			Hot flush (0.2%) Shock hemorrhagic (0.2%)
Respiratory, thoracic and mediastinal disorders		Cough (0.7%)	Pleural effusion (0.2%) Rhinorrhea (0.2%) Nasal congestion (0.2%) Wheezing (0.2%) Pulmonary edema (0.2%)
Gastrointestinal disorders		Diarrhea (0.9%) Vomiting (0.5%)	Nausea (0.2%) Stomatitis (0.2%) Ascites (0.2%) Gastric varices hemorrhage (0.2%) Gastrointestinal hemorrhage (0.2%) Hemorrhoidal hemorrhage (0.2%) Feces discolored (0.2%)
Hepatobiliary disorders		Deterioration of hepatic cirrhosis (0.5%)	
Skin and subcutaneous tissue disorders	Rash (2.1%) Pruritus (1.7%)	Henoch-Schonlein purpura (0.5%) Drug eruption (0.5%)	Erythema (0.2%) Pruritus generalized (0.2%) Purpura (0.2%) Eczema (0.2%)
Musculoskeletal and connective tissue disorders			Pain in extremity (0.2%) Buttock pain (0.2%)
General disorders and administration site conditions	Pyrexia (6.9%)	Injection site pain (0.7%)	Malaise (0.2%) Feeling hot (0.2%) Edema (0.2%)
Investigations			Platelet count decreased (0.2%)

Table 4. Incidences of overall and allergy-related adverse drug reactions during each course

	First course (n = 423)		Second course (n = 314)		Third course (n = 219)		Fourth course (n = 63)		Fifth course (n = 34)		Total (n = 423)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Overall	60	14.2	28	8.9	24	11.0	5	7.9	2	5.9	96	22.
Skin symptoms	19	4.5	2	0.6	2	0.9	0	0.0	0	0.0	23	5.
Respiratory symptoms	1	0.2	3	1.0	3	1.4	1	1.6	1	2.9	6	1.
Pyrexia	20	4.7	8	2.5	5	2.3	1	1.6	1	2.9	29	6.

Table 5. Patients with allergic adverse drug reactions as judged by the investigators

Symptom	Age (years)	Sex	Course	Onset	Outcome
Rash	74	M	1	day 4	Improvement (day 50)
Rash	69	M	1	day 4	Regression (day 9)
Rash	59	M	1	day 5	Regression (day 16)
Rash	74	M	2	day 6	Regression (day 31)
Rash	54	F	1	day 9	Regression (day 31)
Rash	57	M	1	day 14	Regression (day 58)
Rash	73	M	1	day 22	Regression (day 26)
Rash	72	M	1	day 23	Regression (day 35)
Drug eruption	60	M	1	day 3	Regression (day 24)
Drug eruption	66	F	1	day 12	Regression (day 48)
Henoch-Schonlein purpura	56	M	1	day 5	Regression (day 18)
Henoch-Schonlein purpura	63	M	3	day 9	Regression (day 94)
Purpura	53	M	1	day 3	Regression (day 8)
Wheezing	58	M	3	day 1[a]	Regression (day 27)

[a] Did not occur immediately after rHSA administration

Table 6. Abnormal changes in laboratory test values bearing a relationship to rHSA

	Incidence		
	≥1%	0.5% to <1%	<0.5%
Hematological test	Hemoglobin decreased (1.9%) Eosinophil increased (1.9%) Neutrophil increased (1.9%) RBC decreased (1.7%) Hematocrit decreased (1.7%) Lymphocyte decreased (1.7%) Platelet decreased (1.4%)	WBC decreased (0.9%) WBC increased (0.7%) Eosinophil decreased (0.5%) Basophil increased (0.5%)	Neutrophil decreased (0.2%) Lymphocyte increased (0.2%) Monocyte increased (0.2%) Monocyte decreased (0.2%)
Biochemical test	BUN increased (2.4%) Creatinine increased (1.4%) Total bilirubin increased (1.2%)	Direct bilirubin increased (0.9%) Al-P increased (0.7%) Potassium decreased (0.7%) Potassium increased (0.5%)	Total protein increased (0.2%) Total cholesterol decreased (0.2%) AST increased (0.2%) ALT increased (0.2%) LDH increased (0.2%) Al-P decreased (0.2%) Sodium decreased (0.2%) Chloride decreased (0.2%)
Urinalysis	Urinary occult blood aggravated (2.9%) Urine protein aggravated (2.4%) Urine urobilinogen aggravated (1.4%)	Urinary sugar aggravated (0.5%)	

RBC, red blood cell count; WBC, white blood cell count; BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase

Table 7. Incidences of overall and allergy-related adverse drug reactions in specific IgE-positive and -negative patients

	Specific IgE-positive patients (n = 24)		Specific IgE-negative patients (n = 399)		
	No.	%	No.	%	P value (Fisher)
Overall	6	25.0	90	22.6	0.8026
Skin symptoms	1	4.2	22	5.5	1.0000
Respiratory symptoms	0	0.0	6	1.5	1.0000
Anemia	2	8.3	27	6.8	0.6755

Table 8. Incidences of overall and allergy-related adverse drug reactions in patients with "≥50% increase" vs. "no increase" in specific IgG antibody titers

	≥50% increase (n = 88)		No increase (n = 335)		P value
	No.	%	No.	%	(Fisher)
Overall	22	25.0	74	22.1	0.5690
Skin symptoms	3	3.4	20	6.0	0.4374
Respiratory symptoms	0	0.0	6	1.8	0.3519
Pyrexia	7	8.0	22	6.6	0.6379

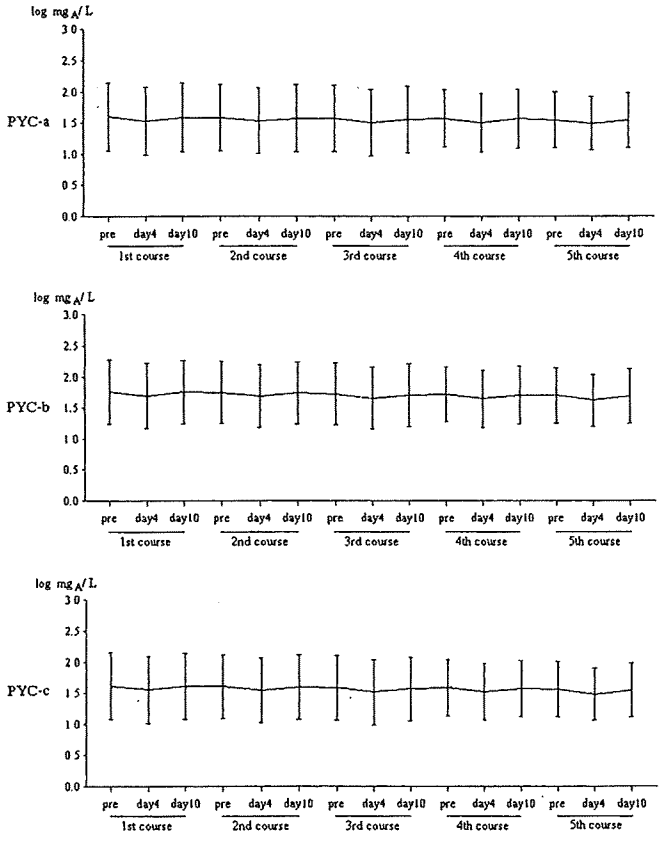

Fig. 1. Time course of specific IgG antibody titers against *Pichia* yeast components. Mean logarithmic values and SDs of specific IgG antibody titers against PYC-a, -b, and -c are shown

although their mean logarithmic values did not change during the repeated administration (Fig. 1A–C).

No difference in the incidence of overall and allergy-related ADRs was observed between the 88 patients with an increase in specific IgG antibodies and the remaining 335 patients with no increase (Table 8).

Efficacy

In all courses, the serum albumin level and colloid osmotic pressure increased significantly after treatment ($P < 0.0001$) (Table 9). In the first, second, and third courses, body weight decreased significantly after treatment ($P < 0.0001$); it also decreased in the fourth and fifth courses, but not significantly (Table 9).

Discussion

pHSA preparations are used to treat various diseases, including liver cirrhosis, hemorrhagic shock due to trauma and surgery.[10,11] In addition to medical use, they are widely used as stabilizers for pharmaceutical products. rHSA produced by *Pichia pastoris* has been developed free from risk of infection with unknown viruses or prions, and as the supply is stable, it is expected to become a useful substitute for pHSA preparations.

Although highly purified rHSA has been shown to be identical to pHSA in structure as well as in physicochemical and immunochemical properties, it is possible for rHSA to contain a very small amount of *Pichia* yeast components. In general, once a patient is sensitized to an antigen, subsequent exposure will elicit a more rapid and severe response than the initial exposure. Therefore, for assessment of safety, clinical study of repeated administration of rHSA is needed to confirm whether small quantities of *Pichia* yeast components cause allergic reactions.

In the present study, rHSA was administered to 423 patients with ascites or edema due to hepatic cirrhosis. No serious allergic ADRs were observed, nor any increase in the incidence of overall or allergy-related ADRs, as defined by the sponsor, during repeated administration. In all 14 patients who experienced allergic ADRs, as judged by the investigators, after rHSA administration, no specific IgE antibody titers against *Pichia* yeast components were detected. While type I allergic reactions via specific IgE antibodies normally occur immediately after exposure to the antigen, this was not the case in these 14 patients; not even wheezing, the earliest observed ADR, occurred immediately after administration (Table 5). It was therefore concluded that none of the allergic ADRs in these patients were type I reactions triggered by rHSA administration. In

Table 9. Changes in serum albumin level, colloid osmotic pressure, and body weight after repeated administration

Parameter	Course	*n*	Day 1 (pretreatment) (mean ± SD)	Day 4 (mean ± SD)	Difference (mean ± SD)	*P* value (paired *t* test)
Serum albumin level (g/dl)	1	423	2.6 ± 0.3	3.3 ± 0.4	0.7 ± 0.3	<0.0001
	2	310	2.8 ± 0.4	3.5 ± 0.4	0.7 ± 0.2	<0.0001
	3	217	2.8 ± 0.4	3.4 ± 0.4	0.6 ± 0.3	<0.0001
	4	63	2.9 ± 0.4	3.6 ± 0.4	0.6 ± 0.3	<0.0001
	5	34	3.0 ± 0.3	3.6 ± 0.4	0.6 ± 0.2	<0.0001
Colloid osmotic pressure (mmHg)	1	423	18.3 ± 2.7	21.0 ± 3.2	2.6 ± 2.3	<0.0001
	2	310	19.5 ± 2.8	22.1 ± 3.2	2.6 ± 2.4	<0.0001
	3	217	19.4 ± 3.0	21.8 ± 3.3	2.4 ± 2.7	<0.0001
	4	63	20.0 ± 3.3	22.4 ± 3.5	2.4 ± 2.5	<0.0001
	5	34	19.7 ± 2.5	22.5 ± 2.9	2.8 ± 2.1	<0.0001
Body weight (kg)	1	423	60.9 ± 11.4	60.3 ± 11.3	−0.7 ± 1.4	<0.0001
	2	311	60.1 ± 11.7	59.7 ± 11.6	−0.4 ± 1.2	<0.0001
	3	217	61.2 ± 11.2	60.6 ± 11.2	−0.6 ± 1.2	<0.0001
	4	61	60.5 ± 10.2	60.3 ± 10.1	−0.2 ± 1.1	0.1899
	5	34	60.1 ± 10.3	59.9 ± 10.0	−0.2 ± 1.0	0.1739

addition, none of these patients experienced eosinophilia. However, the mechanisms for these ADRs were unknown.

Screenings were conducted in 472 patients, and 39 patients were specific IgE positive before treatment. The incidence of specific IgE-positive subjects in our cirrhosis patients was therefore 8.3% (39/472). Of these 39 patients, 19 were included in this study. There are also five patients in whom specific IgE antibodies were detected after treatment, but not before treatment. However, no allergic reactions were observed in any patient with similar specific IgE antibody levels to those of the two patients who experienced serious allergic ADRs in the American study. This result may be because the products used in the American study were more antigenic than those used in the present study, as shown by PCA test.

In general, as specific IgE antibody titers increase, the risk of allergy increases.[12,13] However, no difference in the incidence of overall or allergy-related ADRs was observed between the 24 patients in whom specific IgE antibodies were detected before or after treatment, and the remaining 399 patients in whom they were not detected, indicating that the ADR incidence had no relationship with the specific IgE antibody titers. Thus, there was no tendency toward an increased risk of allergy in patients with higher specific IgE antibody titers. In addition, no change in specific IgE antibody titers after treatment with rHSA was observed in 414 of 423 patients, suggesting that the *Pichia* yeast-derived impurities in this product may be less antigenic.

Generally, once a patient is sensitized to an antigen, specific IgG antibodies increase exponentially. In the present study, 422 of 423 patients had already acquired specific IgG antibodies against *Pichia* yeast components before treatment. It was speculated that specific antibodies against *Pichia* yeast components might have cross-reacted with another yeast, such as *Saccharomyces cerevisiae*, consumed in foods. In 88 patients, antibody titers increased by 50% or more after treatment; however, no patients showed increases of tenfold or more. Mean logarithmic values of specific IgG antibody titers did not change after repeated administration. No difference in the incidence of overall or allergy-related ADRs was observed between the 88 patients with an increase in specific IgG antibody titers and 335 with no increase, suggesting allergic symptoms were not related to these titers.

Thus, assessment of the relationship between specific IgE and IgG antibody titers and symptoms after repeated administration revealed that the ADR symptoms were not related to these specific antibodies, suggesting that this product might possess little antigenicity derived from *Pichia* yeast components.

On the other hand, certain nonallergic ADRs might be attributable to the physiological effects of albumin, including hemorrhage-related events induced by increased circulating plasma volume, and hepatic encephalopathy, which might have resulted from intravascular dehydration induced by the enhanced effect of the diuretics. As albumin preparations are administered to patients with various complications, it is important to administer rHSA with due attention to not only allergic reactions but also any change in circulating plasma volume after administration.

It was concluded that rHSA caused no serious allergic reactions during or after repeated administration to liver cirrhosis patients with ascites or edema. Accumu-

lation of additional data for patients with various diseases by using postmarketing surveys is needed to confirm further the safety of rHSA.

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Special Report

Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts

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Aim: We aimed to identify the candidates for antiviral therapy, among patients who are hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT), focused on the inhibition of hepatocellular carcinoma (HCC).

Methods: Four hundred and sixty-four HCV carriers with normal serum ALT and 129 HCV carriers with persistently normal ALT (PNALT) and platelet (PLT) counts ≥150 000/μL who received liver biopsies were enrolled. HCV carriers with normal serum ALT were divided into four groups according to their ALT levels (≤30 U/L or 31–40 U/L) and PLT counts (≥150 000/μL or <150 000/μL).

Results: In 129 HCV carriers with PNALT, the rate of progression of fibrosis stage was 0.05/year and no HCC was detected during the follow up for 10 years. Approximately 20% of patients with ALT ≤40 U/L and PLT counts ≥150 000/μL were at stage F2–3; however, approximately 50% of patients with ALT ≤ 40 U/L and PLT counts <150 000/μL were at stage F2–4. An algorithm for the management of HCV carriers with normal serum ALT was advocated based on ALT and PLT counts.

Conclusion: The combination of ALT and PLT counts is useful for evaluating the fibrosis stage in HCV carriers with normal serum ALT. Most patients with PLT counts <150 000/μL are candidates for antiviral therapy, especially those with ALT levels ≥31 U/L when we focus on the inhibition of the development of HCC.

Key words: antiviral therapy, chronic hepatitis C, hepatitis C virus carriers, normal serum aminotransferase, platelet count

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) caused by hepatitis C virus (HCV) infection usually develops in patients with advanced chronic hepatitis (CH) or liver cirrhosis. The antiviral treatment for chronic hepatitis C (CH-C) is useful for inhibiting hepatic inflammation and progression of hepatic fibrosis, and consequently the development of HCC.[1–6]

Serum aminotransferase (ALT) levels are within the normal ranges in 20–40% of patients with chronic HCV infection,[7–11] defining the upper limit of normal serum ALT as ≤40 U/L. Significant hepatic fibrosis (≥F2 by the METAVIR classification) has been demonstrated in 5–30% of such patients.[9,12–16] We reported previously

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that HCV carriers with persistently normal ALT (PNALT) had histological features ranging from normal to minimal CH[17,18]; they showed slow progression of liver fibrosis and were at very low risk of developing HCC.[18]

The National Institute of Health Consensus Development Conference reported that HCV carriers with normal serum ALT are candidates for antiviral therapy.[19] A controlled study for the treatment of HCV carriers with PNALT with pegylated interferon alpha and ribavirin (PEG-IFN/Riba) for 48 weeks led to the eradication of HCV RNA in 40% of patients with genotype 1 and high viral load,[20] which is similar to the results of CH-C patients with elevated ALT levels.[21,22] However, it remains controversial whether these patients are candidates for antiviral therapy because of the limited efficacy of treatment, post-treatment flare-up, various side-effects, high cost of treatment, and their good prognoses.

In many Western countries, the upper limits of normal serum ALT are below 40 U/L;[23] however, a recent report from Italy demonstrated that the upper limit in healthy individuals was less than 30 U/L for men and 19 U/L for women.[24] We attempted to draft therapeutic guidelines for the treatment of HCV carriers with normal serum ALT. The biochemical and histological analyses were performed in HCV carriers with serum ALT levels below 40 U/L. These patients were divided into two groups based on ALT levels and then further divided into two subgroups according to their platelet (PLT) counts. We proposed an algorithm for the treatment of HCV carriers with normal serum ALT, taking into consideration the risk of progression to cirrhosis and the development of HCC. The present study demonstrated that the ranges of serum ALT and PLT counts are useful for deciding the indication of antiviral therapy for HCV carriers with normal serum ALT.

METHODS

Eligibility and definition

TWELVE HEPATOLOGISTS BELONGING to the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis, supported by the Ministry of Health, Labour and Welfare of Japan, which was settled on April 2004, participated in the study. Hiromitsu Kumada (Toranomon Hospital, Tokyo, Japan) serves as a chief and Takeshi Okanoue served as a researcher responsible for drafting the guidelines for the treatment of HCV carriers with normal serum ALT. In the present study, we tentatively defined the upper limit of the normal serum ALT as ≤40 U/L.

Patients with hepatitis B virus surface antigen, previous IFN treatment, history of heavy alcohol abuse, antinuclear antibody or antismooth muscle antibody, overt diabetes mellitus, or obesity (body mass index; ≥25 kg/m^2) were excluded from the study.

All of the patients underwent liver biopsy (≥2.0 cm in length) within 6 months prior to antiviral therapy, at which time their serum ALT levels were ≤40 U/L. Informed consent was obtained from every patient prior to liver biopsy and antiviral therapy.

Another study was conducted from January 1990 to August 2004 at Kyoto Prefectural University of Medicine (Kyoto, Japan). HCV carriers with PNALT were defined by serum ALT levels ≤30 U/L on at least three different occasions over a 12-month period and PLT counts ≥150 000/μL as reported previously.[18]

Study design

Among the 580 HCV carriers with normal serum ALT (≤40 U/L), 116 patients were excluded from the study because of insufficient data. Thus, 464 patients who received antiviral therapy from 1995 to 2004 were enrolled in this study (Table 1). Formalin-fixed liver specimens were stained with hematoxylin–eosin, and with Masson's trichrome. The liver specimens ($n = 262$) were also stained with Perls' Prussian blue to study hepatic iron loading. The histological findings were scored according to the classification proposed by Desmet *et al.*[25] and Ishak *et al.*[26] Steatosis was defined as fat droplets in >10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0–4+, based on the scoring system of MacSween *et al.*[27]

The serum ALT, blood glucose level, immunoreactive insulin (IRI), serum ferritin, PLT count, serum hyaluronic acid, amount of serum HCV RNA, and the HCV genotype were examined. The homeostasis model assessment–insulin resistance was calculated as follows: plasma fasting glucose (mg/dL) × IRI (ng/mL) ÷ 405. The serum HCV RNA levels were determined using an Amplicor GT HCV monitor (Roche Diagnostic Systems, Tokyo, Japan). HCV genotype 1 (G1) and 2 (G2) were determined by a serologic genotyping assay.[28] G1 and G2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds *et al.*[29]

All the patients received IFN monotherapy or IFN/Riba combination therapy for 12–36 weeks. The average

Table 1 Baseline of hepatitis C virus patients with normal serum aminotransferase (ALT) received antiviral therapy

	ALT ≤ 30 U/L (group A)	ALT 31–40 U/L (group B)	*P*-value
No. patients	255	209	
Age	51.6 ± 13.0	53.5 ± 13.2	0.548*
Sex (male/female)	112/143	117/92	0.01**
BMI (kg/m^2)	21.6 ± 2.9	22.8 ± 3.0	<0.001*
HOMA-IR	2.5 ± 3.2	5.2 ± 6.5	0.093*
Genotype: 1/2/others	127/127/1	112/96/1	0.881**
Viral load: low/high	138/117	99/110	0.203**
G1 (low/high)	114/125		
G2 (low/high)	161/62		
Histology			
F stage (0/1/2/3/4)	29/166/48/11/1	22/122/57/6/2	0.169**
Grade (0/1/2/3)	25/187/41/2	7/159/43/0	0.046**
Fatty change† 0–1/2–4	232/23	161/48	0.033**
Iron load‡ 0/1–4	101/15	97/19	0.458**
Ferritin (ng/mL)	83.9 ± 103.7	118.8 ± 135.3	0.006*
PLT count (/μL)	19.2 ± 5.4	18.4 ± 6.1	0.059*
≥150 000/<150 000	204/51	141/68	0.002**
Hyaluronate (ng/mL)	60.8 ± 73.7	69.1 ± 73.0	0.249*
Duration of antiviral therapy (weeks)	25.6 ± 12.0	26.1 ± 12.1	0.297*
Effects of therapy			
SVR/non-SVR	142/113	99/110	0.075**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in sex distribution ($P = 0.01$), BMI ($P = 0.01$), frequency of steatosis ($P = 0.033$), serum ferritin level ($P = 0.006$), grade of hepatic inflammation ($P = 0.046$), incidence of fatty change ($P = 0.033$), serum ferritin level ($P = 0.006$), and the incidence of low PLT counts ($P = 0.002$) between groups A and B. Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; PLT, platelet; SVR, sustained viral responders.

duration of therapy between 1995 and 2003 was 26 weeks for IFN monotherapy and 24 weeks for IFN/Riba combination therapy. In principle, 6–10 MU IFN was administered daily for 2 weeks and three times per week subsequently. The daily dosage of ribavirin was 600–1000 mg depending on body weight. Sustained viral responders (SVR) were defined as patients who were negative for serum HCV RNA 6 months after the completion of antiviral therapy.

All of the patients were divided into two groups (group A: ALT ≤ 30 U/L, group B: 31 U/L ≤ ALT ≤ 40 U/L) which were further divided into two subgroups based on PLT counts: group A-1 and B-1 (PLT counts ≥150 000/μL) and groups A-2 and B-2 (PLT counts <150 000/μL).

One hundred and twenty-nine HCV carriers with PNALT were enrolled to determine their long-term prognosis. These patients showed normal serum ALT levels (≤30 U/L) over a 12-month period on least three different occasions (PLT counts ≥150 000/μL, and body mass index [BMI] <25 kg/m^2). Thirty-nine patients received serial liver biopsies. The mean follow-up period of the 129 patients was 7.2 ± 3.2 years on 15 November 2006.

Statistical analyses

Data are expressed as mean ± SD. We compared continuous variables using the Mann–Whitney *U*-test. A frequency analysis and comparison between the groups were performed using the χ^2-test or Fisher's exact test and the Mann–Whitney *U*-test. ANOVA and Tukey's HSD procedure was used to determine the difference between multiple groups. All tests were two-tailed and *P*-values of less than 0.05 were considered significant. All statistical analyses were performed using Statistical Package of Services Solutions software, version 11.0 (SPSS, Chicago, IL, USA).