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
Others	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

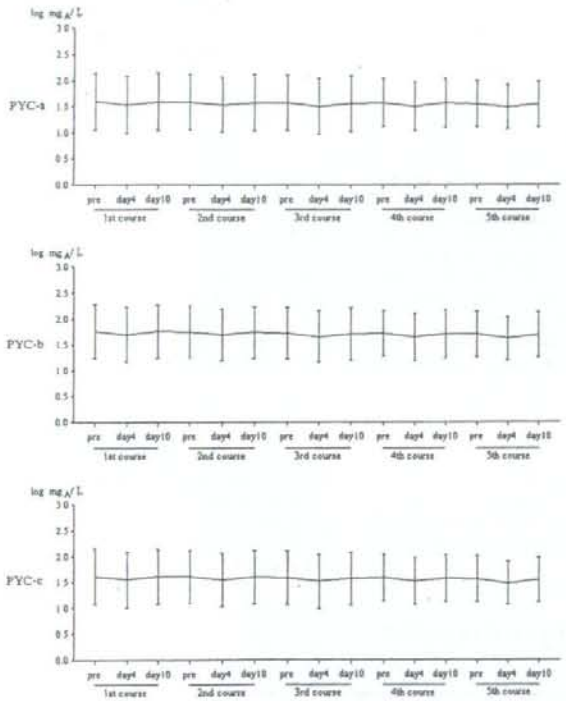

Fig. 1. Time course of specific IgG antibody titers against *Pichia* yeast components. Mean logarithmic values and SDs of specific IgG antibody titers against PYC-a, -b, and -c are shown

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/m²)	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/m²). 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).

Table 2 Baseline of hepatitis C virus patients with less than 30 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/μL (group A-1)	PLT < 150 000/μL (group A-2)	*P*-value
No. patients	204	51	
Age	48.4 ± 12.7	58.7 ± 7.5	<0.001*
Sex (male/female)	90/114	22/29	1.000**
BMI (kg/m²)	21.6 ± 3.0	21.3 ± 2.4	0.514*
HOMA-IR	2.8 ± 3.5	1.2 ± 0.8	0.598*
Genotype: 1/2/others	101/101/2	25/26/0	0.952**
Viral load: low/high	112/92	26/25	0.574**
Histology			
F stage (0/1/2/3/4)	29/142/27/6/0	1/25/21/3/1	<0.001**
Grade (0–1/2,3)	179/25	33/18	<0.001**
Fatty change† 0–1/2–4	188/16	44/7	0.582**
Iron load‡ 0/1–4	82/12	17/3	0.762**
Ferritin (ng/mL)	86.0 ± 112.1	73.9 ± 46.6	0.204*
PLT count (/μL)	21.0 ± 4.4	12.1 ± 2.5	<0.001*
Hyaluronate (ng/mL)	41.8 ± 56.1	112.5 ± 109.9	<0.001*
Duration of antiviral therapy (weeks)	25.7 ± 10.3	27.0 ± 9.9	0.503*
Effects of therapy			
SVR/non-SVR	115/89	27/24	0.66**

**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 age ($P < 0.001$), distribution of F stage ($P < 0.001$), grade of inflammatory activity ($P < 0.001$), PLT count ($P < 0.001$), and serum-hyaluronic acid ($P < 0.001$) between groups A-1 and A-2. Frequency of F2–4 patients was 16.2% in group A-1 and 51.6% in group A-2. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; PLT, platelet counts; SVR, sustained viral responders.

RESULTS

Demographic, clinical, and histological features of 464 HCV carriers with normal serum ALT

THE CHARACTERISTICS OF the 464 HCV carriers with normal serum ALT are shown in Table 1. There were significant differences in sex, frequency of steatosis, serum ferritin levels, BMI, and the incidence of low PLT counts (<150 000/μL) between groups A and B.

There were significant differences in age, fibrosis (F) stage, inflammatory activity, PLT counts, and serum hyaluronate between groups A-1 and A-2 (Table 2). The frequency of stage F2–4 patients was 16.2% in group A-1, and 49.0% in group A-2 (Table 2). In group B, there were significant differences in age, F stage, PLT counts, and serum hyaluronate between groups B-1 and B-2 (Table 3). There were no F4 patients in group A-1 and B-1, and the frequency of F3 patients was very low compared with those in groups A-2 and B-2 (2.6% *vs* 7.6%). The PLT counts decreased in proportion to the progression of liver fibrosis as follows; F0 ($n = 51$); $20.7 \pm 5.2 \times 10^4$/μL, F1 ($n = 288$); $19.8 \pm 5.6 \times 10^4$/μL, F2 ($n = 105$); $16.9 \pm 5.3 \times 10^4$/μL, F3 ($n = 17$); $15.9 \pm 4.6 \times 10^4$/μL, and F4 ($n = 3$); $11.3 \pm 3.8 \times 10^4$/μL.

Of the 464 patients, the frequency of the F0–1 stages was 80.1% and that of the F2–4 stages was 19.9% in patients with PLT counts ≥150 000/μL, and it was 50.4% and 49.6%, respectively, in patients with PLT counts <150 000/μL. In patients with PLT counts $\geq 17.0 \times 10^4$/μL, 80.8% were in stages F0–1 and 19.2% were in stages F2–4, and in patients with PLT counts $< 17.0 \times 10^4$/μL, 60.1% were in stages F0–1 and 39.9% were in stages F2–4.

The SVR rates of IFN therapy were 52.4% in F0–1 patients, 49.5% in F2–4 patients ($P = 0.896$ by Fisher's exact test), and 58.0% and 43.8% ($P = 0.592$) in IFN/Riba therapy, respectively.

In patients with genotype 1b and high viral load, the SVR rate was 12.5%. The SVR rate in genotype 2 patients was 60.4% in the IFN group and 67.7% in the IFN/Riba combination therapy group.

Table 3 Baseline of hepatitis C virus carriers with 31–40 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/μL (group B-1)	PLT < 150 000/μL (group B-2)	*P*-value
No. patients	141	68	
Age	48.2 ± 11.9	57.9 ± 7.5	<0.001*
Sex (male/female)	80/61	37/31	0.751**
BMI (kg/m²)	22.9 ± 3.1	22.7 ± 2.6	0.08*
HOMA-IR	3.0 ± 2.0	8.2 ± 9.5	0.8.8*
Genotype: 1/2/others	82/58/1	30/38/0	0.095**
Viral load: low/high	64/77	35/33	0.542**
Histology			
F stage (0/1/2/3/4)	17/91/31/2/0	4/30/26/6/2	<0.001**
Grade (0–1/2,3)	116/25	50/18	0.114**
Fatty change† 0–1/2–4	111/30	50/18	0.10**
Iron load‡ 0/1–4	67/12	30/7	0.762**
Ferritin (ng/mL)	114.4 ± 116.1	127.2 ± 167.8	0.869*
PLT count (/μL)	21.5 ± 4.9	12.2 ± 2.1	<0.001*
Hyaluronate (ng/mL)	46.9 ± 35.4	100.7 ± 0.98.1	<0.001*
Administration of IFN (weeks)	26.1 ± 11.9	27.7 ± 11.4	0.983*
Effects of therapy			
SVR/non-SVR	64/77	35/33	0.409**

**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×. In group B, there were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), PLT count ($P < 0.001$), and hyaluronic acid ($P < 0.001$) between B-1 and B-2. Frequency of F2–4 was 23.4% in B-1and 50.0% in B-2, respectively. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; IFN, interferon; PLT, platelet counts; SVR, sustained viral responders.

Demographic, clinical, and histological features of 129 HCV carriers with PNALT

The demographic and clinical features of the 129 HCV carriers with PNALT who were followed up for 7.2 years are shown in Table 4. Normal liver histology was noted in 17 patients, 102 showed minimal to mild CH, and 10 had moderate CH. Steatosis was seen in 7% and iron loading was noted in 12%.[18]

Of the 78 patients followed longer than 7 years (mean follow-up period; 10.4 ± 3.1 years), 11 (14%) had continuously normal ALT (G-1), 43 (55%) showed a transient elevation of ALT (G-2), and 24 (31%) changed to CH with continuously elevated ALT (G-3).

Thirty-nine patients received repeated liver biopsies (2–4 times). Of the 39 patients, six were in G-1, 17 were in G-2, and 16 were in G-3. The intervals between the first biopsy and the last biopsy in these three groups were 7.1, 7.8, and 7.2 years, respectively. The progression of the F stage was noted in two of six in G-1, six of 17 in G-2, and seven of 16 in G-3. The median rates of fibrosis progression per year for these three groups were 0.05, 0.05, and 0.08 fibrosis unit. HCC was not detected in any patients during the follow-up periods.

Guidelines for the antiviral therapy of HCV carriers with normal serum ALT focused on the inhibition of the development of HCC

Considering the risk of progression to liver cirrhosis and the development of HCC, as well as the expected efficacy and various side-effects of antiviral therapy, an algorithm is needed for the management of HCV carriers with normal serum ALT. The progression rate of liver fibrosis stage was 0.05/year in HCV carriers with PNALT. The annual incidence of HCC in CH-C patients has been reported to be 0.5% at stages F0–F1, 1–2% at stage F2, 3–5% at stage F3, and 7% at stage F4.[4]

In principle, follow up without antiviral treatment is recommended for HCV carriers with PNALT (ALT ≤30 U/L) and PLT counts ≥150 000/μL, particularly in older patients (i.e. >65 years old), because over 90% show normal or minimal liver damage with good prognoses. However, antiviral therapy is not contraindicated for such patients since roughly 40% are infected with HCV genotype 2,[18] which suggests a high rate of SVR to the therapy with PEG-IFN/Riba.

As for the indication of antiviral therapy for HCV carriers with normal serum ALT (≤40 U/L), the PLT

Table 4 Characteristics of 129 HCV carriers with persistently normal ALT who received liver biopsy

	$n = 129$	Follow up over 5 years ($n = 78$)
Follow-up period (years)	7.2 ± 3.2	10.4 ± 3.1
Age (years)	48 (21–77)	45 (29–71)
Male ($n = 24$)	49.8 ± 16.4	42.3 ± 14.9
Female ($n = 105$)	47.2 ± 12.5	46.6 ± 11.6
Sex (male/female)	24/105	10/68
ALT (U/L)	8–30	9–30
Male ($n = 24$)	22.5 ± 5.7	21.1 ± 5.4
Female ($n = 105$)	21.6 ± 4.8	22.3 ± 5.1
PLT ($\times 10^4/\mu L$)	15–31	15–31
Ferritin (ng/mL)	5–225	5–225
Male ($n = 24$)	76.2 ± 53.5	84.6 ± 59.2
Female ($n = 105$)	60.0 ± 43.3	66.6 ± 52.5
HCV genotype	G1 ($n = 58$), G2 ($n = 45$) Mixed and unclassified ($n = 16$)	
BMI (kg/m^2)	16–27	16–27
Male	22.2 ± 1.7	21.9 ± 1.9
Female	21.3 ± 2.2	21.0 ± 2.4

Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; PLT, platelet.

count is a good indicator for discriminating as to whether or not they have minimal to mild fibrosis or moderate to advanced fibrosis. Serum hyaluronate levels were significantly higher in HCV carriers with 31–40 U/L ALT having less than 150 000/μL PLT (Table 3). Advanced hepatic F stage, an elevated ALT level, old age (>65 years old), and sex (male) are important risk factors for the development of HCC.[6,18,30] We advocated an algorithm for such patients (Fig. 1) taking into consideration the risk of the progression to cirrhosis and the development of HCC. Therapy with PEG-IFN/Riba is the first-line treatment; therapy for 48 weeks is recommended for genotype 1 patients with high viral load and 12–24 weeks therapy for genotypes 2 and 1 with low viral load.

DISCUSSION

OUR PREVIOUS STUDY in 129 HCV carriers with PNALT demonstrated a predominance of females, higher frequency of genotype 2, minimal to mild liver histology, and very slow progression of hepatic fibrosis.[18] However, over 30% of these patients advanced to CH-C with elevated ALT levels during the 7-year follow up.

There are many reports concerning the natural course of liver fibrosis in CH-C patients, including those who are HCV carriers with normal serum ALT.[19,31–39] More than half of CH-C patients show progression of F stage from F1 to F2–4 within 10 years, and it was reported that the progression of liver fibrosis in HCV carriers with normal serum ALT was more rapid than was observed in the present study.[23] The main reason for the discrepancy between the report by Puoti *et al.*[23] and our results might be due to the definitions used for the normal range of serum ALT. In our previous study, the patients were HCV carriers with PNALT (ALT ≤ 30 U/L) and PLT counts ≥150 000/μL. On the other hand, the patients in the study by Puoti *et al.* had ALT levels ≤40 U/L, irrespective of PLT counts, in which cirrhotic patients might be included.[23] However, recent studies have demonstrated that normal ALT levels are less than 30 U/L[24] or 25 U/L in men[40] and less than 19 U/L[24] or 22 U/L in women.[40]

The present study demonstrated that the different distribution of hepatic F stage became remarkable when the A and B groups were divided into two subgroups according to their PLT counts. In HCV carriers with ALT levels ≤30 U/L, the frequency of stages F2–3 was 16.2% among those with PLT counts ≥150 000/μL; however, the frequency of stages F2–3 was 49.0% in those with PLT counts <150 000/μL. Conversely, in HCV carriers with ALT levels between 31 and 40 U/L, the frequency of stages F2–4 was 23.4% among those with PLT counts ≥150 000/μL and 50.0% in those with PLT counts <150 000/μL. The PLT count is a useful marker in dis-

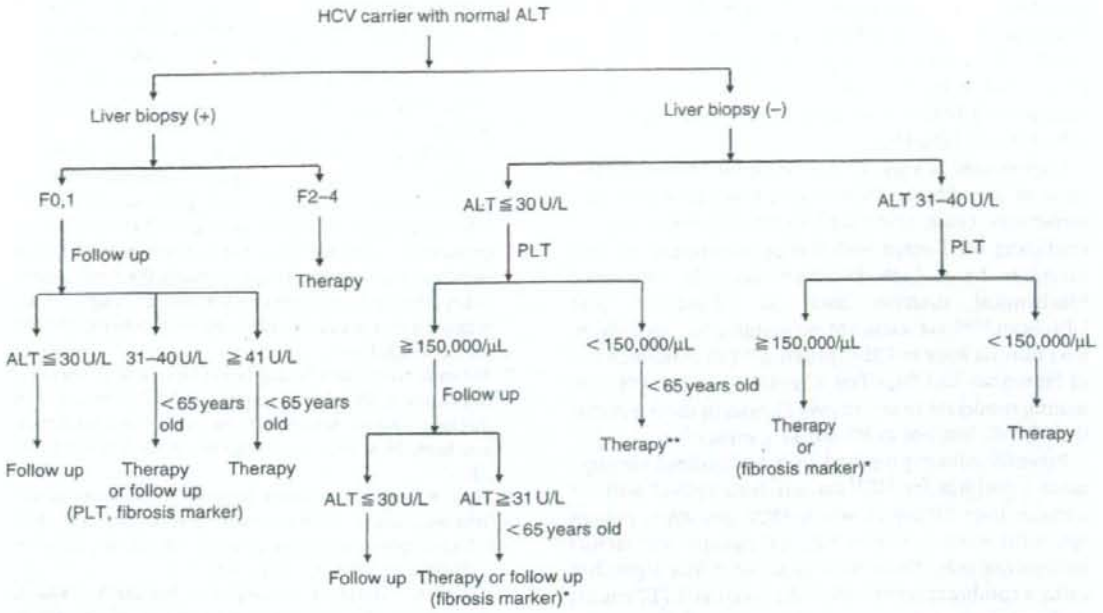

Figure 1 Algorithm for the management of hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT, ≤40 U/L) focused on the inhibition of the development of hepatocellular carcinoma. In patients who underwent liver biopsy, F0 and F1 patients younger than 65 years are candidates for antiviral therapy, especially those with genotype 2 after the elevation of serum ALT levels. In patients who did not undergo liver biopsy, ALT and platelet (PLT) levels are good indicators for determining candidates for antiviral therapy. Older patients (>65 years) and/or patients having uncontrolled hypertension, diabetes mellitus, or anemia should not be treated with pegylated interferon and ribavirin. Combination therapy with pegylated interferon and ribavirin for 48–72 weeks is recommended for patients with genotype 1 and high viral load, and 12–24 weeks therapy is suggested for patients with genotype 2 and genotype 1 with low viral load. *,**Serum fibrosis markers, such as hyaluronate, might be useful to decide whether patients are candidates for antiviral therapy or not.

criminating between stages F0–1 and F2–4 F in HCV carriers with normal serum ALT (≤40 U/L). In the present study, the mean PLT count in F2 and F3 patients was 16.9 ± 5.3 ($\times 10^4/\mu L$) and 15.9 ± 4.6 ($\times 10^4/\mu L$), respectively. The distribution of the F stage was not significantly different between patients with PLT counts $\geq 15 \times 10^4/\mu L$ versus $< 15 \times 10^4/\mu L$ and $\geq 17 \times 10^4/\mu L$ versus $< 17 \times 10^4/\mu L$.

The SVR rate for genotype 1 patients with high viral load treated with either IFN monotherapy or IFN/Riba were 12.5% and 37.7%, respectively. In genotype 2 patients with high viral load, the SVR rate in the present study was better than the data of Japanese CH-C patients with elevated ALT levels in our previous paper.[6] It was not reasonable to compare the SVR rates between HCV carriers with normal serum ALT and CH-C with elevated ALT in the present study, because the total dosage of IFN and the duration of treatment were significantly different.

The annual incidence of HCC is correlated with the progression of liver fibrosis, that is, the stage of liver disease.[2–4,6] Sustained low serum ALT levels are also associated with a lower incidence of HCC.[2,6,41] PEG-IFN/Riba therapy is expensive and induces various side-effects. The present results indicate that most HCV carriers with normal serum ALT (≤40 U/L) and PLT counts ≥150 000/μL have minimal to mild liver damage, indicating a low risk for the progression to cirrhosis and the development of HCC. This was more remarkable in patients with ALT levels ≤30 U/L and PLT counts ≥150 000/μL. However, nearly half of the patients with PLT count <150 000/μL have F2 or F3 F stages, indicating a certain risk for the progression to cirrhosis and the development of HCC. Fibrosis

progression is associated with age, baseline and follow-up ALT levels, inflammatory activity and steatosis in the initial liver biopsy, and alcohol consumption.[42] The present results indicate that most HCV carriers with PNALT have a good prognosis and a low risk of developing HCC.

Liver biopsy is a useful procedure for identifying the stage of liver fibrosis; however, it is invasive and may sometimes cause complications.[43,44] The error rate of predicting the F stage with this procedure can be estimated to be as high as 20%.[45] Recently introduced biochemical markers, such as FibroTest,[46] and FibroScan,[47–49] are excellent procedures for identifying liver fibrosis stage in CH-C patients.[50] The combined use of FibroScan and FibroTest is useful for accurately estimating moderate to severe liver fibrosis in most patients with CH-C, but not in F0 and F1 patients.[51]

Recently, Alberti proposed an individualized management algorithm for HCV carriers with PNALT with or without liver biopsy in which HCV genotype, patient age, motivation to receive antiviral therapy, and factors influencing side-effects were included.[52] The algorithm using a combination of serum ALT levels and PLT counts in the present study is simple, but it is useful because it focuses mainly on the inhibition of the progression to cirrhosis and the development of HCC.

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Virological Response in Patients with Hepatitis C Virus Genotype 1b and a High Viral Load

Impact of Peginterferon-α-2a plus Ribavirin Dose Reductions and Host-Related Factors

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Abstract

Background and objective: In Japan the prevalence of the hepatitis C virus (HCV) antibody is highest in the elderly population. Therefore, it is important for elderly patients to undergo interferon (IFN) therapy. In patients with HCV genotype 1b and a high viral load, the sustained virological response (SVR) rate is lower in older compared with younger patients receiving combination antiviral therapy. In addition, inadequate adherence to combination therapy is often seen in elderly patients, and is associated with reduced response rates. The aim of this retrospective analysis was to evaluate the effects of host-related factors (i.e. sex, age, baseline HCV RNA level, bodyweight and fibrosis stage) and peginterferon (PEG IFN)-α-2a plus ribavirin dose reductions on SVR rates.

Methods: A total of 192 treatment-naive patients with a HCV genotype 1b infection and a high viral load were included in the analysis. Patients had been enrolled into a phase III trial of 48 weeks of treatment with PEG IFN-α-2a plus ribavirin or PEG IFN-α-2a plus placebo. All patients were evaluated for effect of drug exposure on SVR. In addition, the impact of host-related factors or dose reductions on SVR was assessed.

Results: Approximately 30% of patients were considered elderly (≥60 years of age). The overall SVR rate was significantly higher in patients treated with combination therapy versus monotherapy (59.4% vs 24.0%, $p < 0.001$). Attainment of an SVR following combination therapy was not influenced by any factor evaluated in the analysis, although elderly males were associated with decreased SVR rates. Younger age (odds ratio [OR] 1.081; 95% CI 1.125, 1.034; $p = 0.0009$), lower baseline HCV RNA levels (OR 1.003; 95% CI 1.006, 1.001;

p = 0.006) and a severe fibrosis stage (F3/4) [OR 6.194; 95% CI 1.037, 37.000; p = 0.0455] significantly increased the likelihood of achieving an SVR with monotherapy. In the combination therapy group, patients maintaining a full dosage schedule of PEG IFN-α-2a and ribavirin and those requiring dose reductions of either study drug had similar SVR rates (64.5% vs 61.9%). However, the SVR rate was reduced to 33.3% among patients who discontinued combination therapy. Three out of the 31 patients who received the full dosage schedule were elderly patients. In addition, of the 15 patients who discontinued combination therapy, three were <50 years of age and six were ≥60 years of age. The SVR rate was reduced in patients with cumulative PEG IFN-α-2a and ribavirin doses of <60%; the majority of these patients were elderly.

Conclusion: The attainment of an SVR following PEG IFN-α-2a plus ribavirin combination therapy was not influenced by any of the host-related factors evaluated in this analysis, although elderly males were associated with a decreased SVR rate. Younger age, male sex and lower baseline HCV RNA levels significantly increased the likelihood of achieving an SVR with monotherapy. In addition, dose reductions appeared to have a negative impact on SVR in elderly patients. Therefore, it is important to minimize PEG IFN-α-2a and ribavirin dose reductions by effectively managing treatment-related adverse events in elderly patients.

Introduction

In Japan, the prevalence of the hepatitis C virus (HCV) antibody is highest in the elderly population. In a recent analysis,[1] the average age of HCV-positive patients in Japan was found to be greater than that of US patients by approximately 20 years. Results of the analysis suggested that the introduction of HCV into the Japanese population occurred >100 years ago, followed by wide dissemination in the 1930s and 1940s. In contrast, HCV was introduced into the US 100 years ago, followed by wide dissemination in the 1960s. This extended period of exposure to HCV was the likely reason for the considerably higher prevalence of hepatocellular carcinoma in Japan.

To date, it is unclear if genetic and/or environmental factors have an influence on the incidence of hepatocellular carcinoma in Japan. The duration of HCV infection appears to be an important factor for the development of hepatocellular carcinoma, although the age of patients with post-transfusion HCV has been reported to be a significant factor, regardless of the duration of exposure to HCV.[2] Therefore, it appears to be important for elderly patients to undergo interferon (IFN) therapy in the absence of serious complications such as uncontrolled hypertension or insulin-dependent diabetes mellitus.

Combination therapy with peginterferon (PEG IFN)-α-2a plus ribavirin was found to be more effective than PEG IFN-α-2a monotherapy in Japanese patients with HCV genotype 1b.[3] However, a recent study showed that sustained virological response (SVR) rates were lower in older (≥40 years of age) compared with younger patients with HCV genotype 1b and a high viral load.[4] In addition, inadequate adherence to combination therapy with IFN-α-2b and ribavirin was independently associated with increasing patient age and a reduction in SVR response rates.[5] There are insufficient numbers of clinical trials evaluating the use of PEG IFN plus ribavirin in elderly patients, and an effective dose and treatment period has not been established.

The aim of this retrospective analysis was to investigate the effects of host-related factors (i.e. sex, age, baseline HCV RNA level, bodyweight and fibrosis stage) and PEG IFN-α-2a plus ribavirin

dose reductions on SVR rates in patients with a difficult-to-treat form of chronic hepatitis C.

Patients and Methods

We retrospectively analysed data from a phase III, randomized, double-blind clinical trial conducted at 43 Japanese centres between June 2002 and September 2004.[3]

Patients

A total of 192 treatment-naive patients were included in the analysis. Inclusion criteria were Japanese adults aged ≥20 years with an HCV genotype 1b infection, a serum HCV RNA level of $\geq 1 \times 10^5$ IU/mL, an elevated serum alanine aminotransferase (ALT) level of ≥45 IU/L within 6 months of screening, and chronic hepatitis C confirmed by liver biopsy. Patients were excluded if they had neutropenia (<1500 neutrophils/mm^3), leucopenia (<3000 cells/mm^3), thrombocytopenia (<90 000 platelets/mm^3), anaemia (haemoglobin <12 g/dL), a hepatitis B virus co-infection, decompensated liver disease, organ transplant, a creatinine clearance <50 mL/min, poorly controlled psychiatric disease, poorly controlled diabetes, malignant neoplastic disease, severe cardiac or chronic pulmonary disease, immunologically mediated disease, or retinopathy.

Study Design

Patients were randomized according to a 1 : 1 ratio to 48 weeks of treatment with subcutaneous PEG IFN-α-2a (Pegasys®, Roche, Tokyo, Japan)[1] 180 μg/week in combination with either twice daily oral ribavirin tablets (Copegus®, Roche, Basle, Switzerland) or placebo, followed by 24 weeks of untreated follow-up. The ribavirin dosage was 600, 800 or 1000 mg/day in patients with a bodyweight of ≤60, 60–80 or >80 kg, respectively; these dosages were based on the currently used dosages of ribavirin in Japan. Patients were stratified according to HCV RNA level.

Virological Methods

Qualitative and quantitative serum HCV RNA assessments were conducted using the Cobas Amplicor HCV Test PCR assay (version 2.0; limit of detection 50 IU/mL) and the Cobas Amplicor HCV Monitor Test (version 2.0; limit of quantitation, 500 IU/mL), respectively. HCV genotyping was performed according to the method described by Okamoto et al.[6] The presence of serum anti-HCV antibodies was not assessed.

Histology

Liver biopsies were taken within 12 months of enrolment. An independent pathologist evaluated, graded and staged liver biopsy specimens according to the Ishak modified hepatic activity index and the new European classification.[7,8]

Assessment of Efficacy

The primary efficacy end point of the study was the SVR rate, which was defined as a HCV RNA level of <50 IU/mL after 24 weeks of untreated follow-up.

Statistics

The Cochran-Mantel-Haenszel test was used to compare treatment groups, with a significance level of $p < 0.05$.

Host-related factors associated with an SVR were evaluated using stepwise and multiple logistic-regression models. The following pretreatment factors were considered: sex, age, bodyweight, serum HCV RNA and fibrosis stage (F1/2: mild/moderate; F3/4: severe/cirrhosis). Factors such as the maintenance of a full dosage schedule, the requirement of dose reductions, and treatment discontinuation were also considered.

All patients receiving at least one dose of study drug were included in the efficacy analysis. Patients without follow-up data were considered not to have attained an SVR.

1 The use of trade names is for product identification purposes only and does not imply endorsement.