achieve an SVR ($P = 0.0047$). The HCV load tended to be lower in patients who achieved an SVR than that in patients who did not achieve a SVR ($P = 0.0514$).

In patients aged ≥65 years, the factors associated with an SVR with combination therapy were determined using multivariate analysis (Table 7). Viral load [$P = 0.015$, odds ratio 1.000 (0.999–1.000)] and genotype [$P = 0.022$, odds ratio 0.268 (0.087–0.830)] were significantly associated with an SVR. Gender [$P = 0.052$, odds ratio 0.410 (0.166–1.009)] tended to be associated with an SVR.

To identify patients aged ≥65 years with genotype 1 (hard-to-treat population) who may benefit from combination therapy, we examined the efficacy of combination therapy according to viral load and gender (Fig. 4). Even among older male patients with high viral loads, patients with viral loads < 2 000 000 IU/ml had a significantly higher SVR than patients with viral loads over 2 000 000 IU/ml [56.7% (17/30) vs. 13.3% (2/15)] ($P = 0.0094$). In contrast, there was no significant difference in the SVR rate between older female patients with viral loads < 2 000 000 IU/ml and those with viral loads over 2 000 000 IU/ml.

To evaluate the ribavirin dose during the first quarter (12 weeks) in each group of genotype 1 patients at two institutions, we calculated the percent intake of the expected dose during the first quarter. The percentage of patients who achieved a drug intake rate over 80% during the first quarter was significantly lower in elderly patients than in younger patients (75.0 vs. 88.4%; $P = 0.0442$). Similarly, the patients who achieved an SVR were more likely to have a drug intake rate over 80% than patients who did not achieve an SVR (91.7 vs. 80.7%; $P = 0.0464$).

Table 7. Multivariate analysis of factors associated with a sustained virological response in patients aged ≥65 years treated with combination therapy

Variable		Odds ratio (95% CI)	*P* value
HCV RNA (kIU/ml)		1.000 (0.999–1.000)	0.015
Genotype	1 vs. 2	0.268 (0.087–0.830)	0.022
Gender	Female vs. male	0.410 (0.166–1.009)	0.052

HCV RNA, hepatitis C virus RNA; kIU, kilo international units; SVR, sustained virological response.

Adverse events

The combination therapy discontinuation rate of patients aged ≥65 years was significantly higher than that of patients aged < 65 years ($P = 0.0003$) (Table 2). Even when excluding genotype 1 cases in which therapy was discontinued because the virus could not be eradicated after 24 weeks, the combination therapy discontinuation rate of patients aged ≥65 years was significantly higher than that of patients aged < 65 years ($P < 0.0001$). Ribavirin discontinuation was higher in older patients ($P = 0.0013$). The reasons for discontinuing combination therapy and the times when therapy was discontinued are shown in Table 8. One case with a serious adverse effect occurred in each group: insulin-dependent diabetes mellitus in the younger group and bleeding from duodenal varices in the older group. The discontinuation rate because of general fatigue or anaemia was higher in older patients than that in younger patients [5.22% (6/115) vs. 1.90% (9/476) ($P = 0.0418$) and 5.22% (6/115) vs. 0.63% (3/476) ($P = 0.0024$) respectively].

Discussion

It is important to eradicate HCV by IFN to reduce the risk of HCC (4, 5). In addition, IFN reportedly reduces liver-related mortality in chronic hepatitis C patients over age 60 years old (11, 21, 22). However, these findings are based on studies of IFN monotherapy. The present study examined the effect of a combination of ribavirin and peginterferon. Ribavirin has been used in combination with IFN or peginterferon to treat chronic hepatitis C, and this combination therapy has been reported to be more effective than IFN monotherapy in eradicating

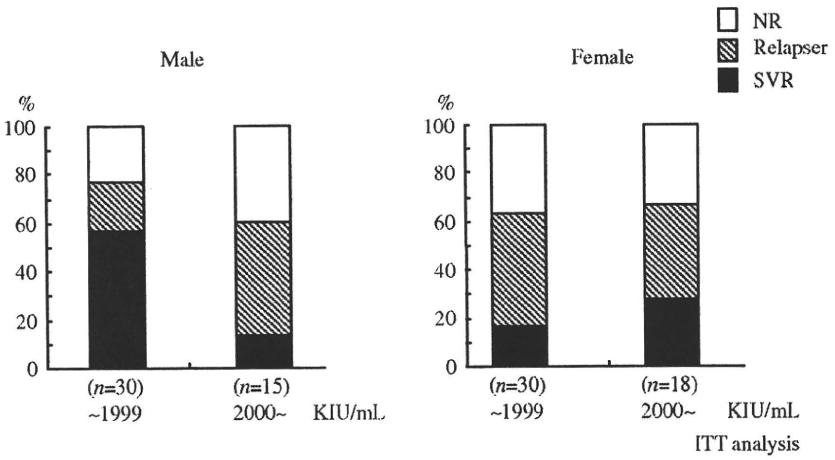

Fig. 4. A virological response to combination therapy according to virus load and gender of older patients with genotype 1. ITT, intention-to-treat; kIU, kilo international units; NR, nonresponder; SVR, sustained virological response.

Table 8. Reasons for discontinuing combination therapy

Reason	Number	Weeks after starting treatment	Reason	Number	Weeks after starting treatment
Patients aged < 65 years (n = 476)			*Patients aged ≥ 65 years (n = 115)*		
Fatigue	9	4, 8, 8, 10, 13, 20, 25, 33	Fatigue*	6	1, 4, 6, 8, 19, 32
Depression	7	1, 2, 4, 8, 13, 15, 18	Anaemia*	6	3, 8, 12, 12, 13, 15
Self-discontinuation	6	8, 16, 23, 24, 25, 28	Rash	3	1, 4, 9
Headache	3	2, 36, 37	Depression	2	2, 9
Anaemia	3	4, 11, 24	Jaundice	1	1
Rash	2	18, 25	Fiver	1	7
Hepatocellular carcinoma	2	19, 43	**Bleeding from duodenal varies‡**	**1**	**8**
Bronchitis	1	2	Anorexia	1	10
Alopecia	1	13	Hyperthyroidism	1	15
Progression of diabetes	1	14	Cholecystitis	1	16
Peritonitis due to appendicitis	1	16	Symptoms of Parkinson's disease	1	16
Fundal hemorrhage	1	17	Suspicion of interstitial pneumonia	1	20
Pneumonia	1	18	Gastric cancer	1	21
Body weight loss	1	22	Hepatocellular carcinoma	1	21
Vertigo	1	25			
Elevation of TSH	1	25			
Unknown	1	25			
Lack of funds	1	27			
Hypothyroidism	1	28			
Gastric cancer	1	38			
Insulin-dependent diabetes mellitus‡	**1**	**44**			
Reappearance of pancreatitis	1	46			
Noneradication of HCV†	34		Noneradication of HCV†	10	

*The ratio of discontinuation was significantly different between the two groups $P < 0.05$.
†Discontinued because the virus could not be eradicated after 24 weeks.
‡Serious adverse effects are shown in bold.

HCV (7–10). However, ribavirin and IFN or peginterferon in combination produce a common adverse effect, i.e. Hb levels decrease in 20–36% of treated patients with chronic hepatitis C, necessitating dose reduction or discontinuation (8, 10, 23, 24). Among elderly patients treated with combination therapy, ribavirin dose reduction is often required, resulting in a reduced SVR in older patients (15, 16). In this study, ribavirin dose reduction was higher in elderly patients than that in younger patients.

Previous studies have reported that there is no significant difference in the efficacy of IFN monotherapy between older and younger patients after normalizing for difference in background clinical characteristics, suggesting that age does not influence the outcome of IFN monotherapy (13, 14).

Adding ribavirin to IFN improves the treatment efficacy. However, ribavirin reduces Hb levels, causing greater dose reductions. Elderly patients with genotype 1 and high HCV loads have a lower SVR rate than younger patients because of a higher ribavirin dose reduction rate and discontinuation rate because of ribavirin-related anaemia (15, 16). We examined chronic hepatitis C patients with a similar background, except for age, and found that combination therapy was comparably effective between patients aged ≥60 years and those aged < 60 years, although the ribavirin discontinuation rate was higher among older patients (17). Similar results were obtained in the chronic hepatitis C patients treated with peginterferon and ribavirin, and positive responses to combination treatment were decreased for genotype 1- or 4-infected patients older than 40 years, but comparable between patients older than 65 and patients aged 40–64 years (18).

However, the background, efficacy and tolerability of peginterferon and ribavirin combination therapy in elderly patients according to gender have not been fully elucidated. Moreover, there are no data identifying which patients will achieve an SVR among older patients. Our previous report examined a 24-week regimen of ribavirin plus interferon therapy and defined advanced age as over 60 years (17). However, currently, the most common treatment protocol is prolonged ribavirin plus peginterferon-α treatment. Moreover, the patient age distribution has shifted to a more advanced age. Therefore, we need to re-evaluate an additional protocol including peginterferon and define advanced age as 65 years. We conducted a multi-institution study to evaluate the efficacy and tolerability of ribavirin plus peginterferon-α in older patients with chronic hepatitis C.

An ITT analysis indicated that the SVR rate in elderly patients was lower than that in younger patients, while a PP analysis showed that the SVR rate in elderly patients was not statistically different from that of younger

patients. These results indicated that when treatment is not discontinued, the SVR rate of elderly patients will be high.

Multivariate analysis showed that baseline age and genotype are factors significantly associated with an SVR. Many studies have shown that baseline viral load and genotype are factors significantly associated with an SVR (8, 24). Age was associated with an SVR and the SVR rate of patients aged ≥65 years was lower than that of patients aged < 65 years (37.4 vs. 51.5%; $P=0.0067$).

Because the SVR differs according to genotype, we classified patients by genotype and compared the SVR rate for both male and female patients. In both male and female patients with genotype 1, the SVR rate decreased with age, and the SVR rate of both patients < 40 years was over 60%. These results were similar to previous studies where the SVR rate of patients < 40 years old was higher than that of another generation (17, 18, 24, 25). In patients ≥65 years old, the SVR rate of female patients was significantly lower than that of male patients [in patients with both genotype 1 and 2, 27.6% (16/58) vs. 47.4% (27/57); $P=0.0284$; in patients with genotype 1, 20.8% (10/48) vs. 42.2% (19/45); $P=0.0261$]. The result that female patients are less likely to achieve an SVR than male patients differs from that of a previous report (25). However, our results are consistent with Sezaki and colleagues, who reported that females have a poorer response to peginterferon and ribavirin combination therapy than males among patients with hepatitis C aged ≥50 years. In the older population, the gender associated with an SVR changes from male to female (26). In both male and female patients with genotype 2, the SVR of all generations was over 60%. A study by Antonucci *et al.* (18)and our previous report suggest that genotype 2 patients have a higher SVR rate, which is age-independent.

We cannot exclude the bias that better candidates were more likely to be selected among older patients than younger patients in the outpatient department. However, regardless of potential bias, elderly patients had low body weight, low Hb levels and an advanced fibrosis stage. Regarding fibrosis, elderly patients are more likely to have a long disease duration as it was reported previously that fibrosis progression was mainly dependent on age and the duration of infection (27). In this study, ITT and PP analyses indicated that the SVR rate did not differ between younger and older patients with F2–F4 fibrosis who needed treatment to prevent liver-related deaths (data not shown).

Several reports suggested that the efficacy of peginterferon and ribavirin combination therapy is lower in elderly patients with genotype 1 than in younger patients, but there are no reports establishing which elderly patients will benefit from this combination therapy. To identify genotype 1 patients ≥65 years old (hard-to-treat population) who will particularly benefit from combination therapy, we examined the efficacy of combination therapy according to viral load and gender. In older male patients with genotype 1 and HCV RNA concentrations < 2 000 000 IU/ml, the SVR rate was over 50%. Based on these results, combination therapy should be considered for male patients with genotype 1 and with HCV RNA concentrations < 2 000 000 IU/ml. Even if the treatment schedules differ between western countries and Japan, age will have to be considered, and viral load will be an important issue when treating elderly patients.

In conclusion, elderly patients in Japan who received combination therapy with peginterferon and ribavirin had a low body weight, low Hb levels and advanced fibrosis. Elderly patients had higher treatment discontinuation rates and lower SVR rates than younger patients. However, an SVR was achieved in over 50% of elderly patients with genotype 2 and in male patients with genotype 1 and HCV RNA concentrations < 2 000 000 IU/ml.

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Appendix 1

The following institutions participated in this study:

- Aihoku Hospital
- Aichi Cancer Center
- Aichi Cancer Center Aichi Hospital
- Aichi Saiseikai Hospital
- Aichi Sannomaru Hospital
- Atsumi Hospital
- Anjo Kosei Hospital
- Ichinomiya Municipal Hospital
- Ichinomiya Municipal Hospital Imaise Branch
- Inazawa City Hospital
- Ogaki Municipal Hospital
- Okazaki City Hospital
- Kainan Hospital
- Kakegawa City General Hospital
- Kamo Hospital
- Kariya Toyota General Hospital
- Gifu Social Insurance Hospital
- KumiaiKosei Hospital
- Aichi Cardiovascular and Respiratory Center
- Showa Hospital
- Tosei General Hospital
- Komaki City Hospital
- Komaki Daiichi Hospital
- Sakashita Hospital
- Saishukan Hospital
- Shizuoka Kosei Hospital
- Shizuoka Saiseikai General Hospital
- Yokkaichi Municipal Hospital
- Holy Spirit Hospital
- Kamiiida daiichi General Hospital
- Daido Hospital
- Chita City Hospital
- Chubu Rosai Hospital
- National Center for Geriatrics and Gerontology
- Tsushima City Hospital
- Tokai Memorial Hospital
- Tokai Sangyo Central Hospital
- Tokai Municipal Hospital
- Tokai Central Hospital
- Tokai Hospital
- Tohno Kousei Hospital

- Toki General Hospital
- Tokoname Municipal Hospital
- Toyota Memorial Hospital
- Toyohashi Medical Center
- Toyohashi Municipal Hospital
- Nakatsugawa Municipal General Hospital
- Nagoya Medical Center
- Nagoya Ekisaikai Hospital
- Nagoya Memorial Hospital
- Nagoya Kyouritu Hospital
- Japanese Red Cross Nagoya First Hospital
- Nishio Municipal Hospital
- Handa City Hospital
- Fukuroi Municipal Hospital
- Fujita Health University Hospital
- Brother Hospital
- Hekinan Municipal Hospital
- Mitsubishi Nagoya Hospital
- Miyoshi Municipal Hospital
- Meijo Hospital
- Meitetsu Hospital
- Yachiyo Hospital
- Yamashita Hospital

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Differences in Viral Kinetics Between Genotypes 1 and 2 of Hepatitis C Virus After Single Administration of Standard Interferon-Alpha

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Hepatitis C virus (HCV) kinetics were determined after a single administration of standard interferon (IFN) according to the HCV genotype that affects the response to antiviral therapy with IFN/peginterferon. A total of 208 patients were investigated. All patients received a single administration of 6 megaunits of standard IFN-alpha. HCV RNA concentration was measured before, and at 24, 48, 72, and 120 hr after administration. Changes in HCV RNA concentration were compared between genotypes. The patient group consisted of 132 patients with genotype 1B, 58 with genotype 2A, and 18 with genotype 2B. In the comparison between genotypes 1 and 2, the reduction in HCV RNA concentration after a single IFN administration was less marked in patients with genotype 1B at both 24 and 48 hr after administration ($P < 0.0001$). In contrast, an increase in HCV RNA concentration during 24–48 or 24–72 hr after a single administration was comparable between genotypes 1 and 2. In the comparison between genotypes 2A and 2B, the reduction in HCV RNA concentration after a single administration was more marked in patients with genotype 2A, despite the similar rate of sustained virologic response to peginterferon and ribavirin combination therapy. The results of the study indicate that the rapid decrease in HCV RNA concentration observed during IFN or peginterferon therapy in patients with genotype 2 appeared to be due to the difference in sensitivity to IFN. Within genotype 2, HCV genotype 2A was more sensitive to IFN than genotype 2B. ***J. Med. Virol. 81:1354–1362, 2009.*** © 2009 Wiley-Liss, Inc.

KEY WORDS: HCV kinetics; HCV genotype; antiviral therapy; standard interferon

INTRODUCTION

Chronic hepatitis C virus (HCV) infection causes an indolent hepatic disease that may cause cirrhosis or hepatocellular carcinoma. Antiviral therapy against HCV with peginterferon (PEG-IFN) and ribavirin can achieve eradication of HCV and contributes greatly to the management of patients with HCV infection.

Therapeutic responses to antiviral therapy with interferon (IFN) have been shown to be influenced by the genotype of HCV infecting the patient in IFN monotherapy, combination therapy with ribavirin, PEG-IFN monotherapy, and most recently in combination therapy with PEG-IFN and ribavirin [Yoshioka et al., 1992; McHutchison et al., 1998; Poynard et al., 1998; Zeuzem et al., 2000; Lindsay et al., 2001; Manns et al., 2001; Fried et al., 2002; Hatziyannis et al., 2004]. The rate of sustained virologic response, in which serum HCV RNA turned negative during treatment and remains so 6 months after the end of treatment and which means the eradication of HCV, is higher for patients with HCV genotype 2 or 3 than for those with HCV genotype 1. In combination therapy with PEG-IFN and ribavirin, which is the current standard antiviral therapy for patients with chronic hepatitis C, the recommended duration of the treatment period is 48 weeks for patients with HCV genotype 1, whereas it is 24 weeks for patients with HCV genotype 2 or 3 [Strader et al., 2004; Dienstag and McHutchison, 2006].

The reason for this significant difference in the response to antiviral therapy between HCV genotypes

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is not fully understood. Which factors contribute to the favorable response to IFN/PEG-IFN for HCV genotype 2 or 3 and to the unfavorable response to IFN/PEG-IFN for HCV genotype 1?

Only two studies by Neumann et al. [2000] and by Karino et al. [2003] analyzed the difference in HCV viral kinetics between genotypes 1 and 2 after the start of monotherapy with standard IFN. These investigators reported that multiple factors contributed to the higher SVR rate to IFN in patients with HCV genotype 2, including higher antiviral effectiveness of IFN in blocking virus production, higher free virion clearance rate, and higher HCV-infected cell death rate. However, their study investigated HCV kinetics during the early part of the treatment period. Under these conditions, the decrease in HCV RNA concentration after the start of IFN therapy could be evaluated, but the increase of HCV RNA after the single administration of IFN could not be evaluated, because subsequent IFN was administered. Therefore, no previous study has evaluated the "pure" response of HCV to IFN according to the genotype.

In the present study, the kinetics of different infecting HCV genotypes were investigated after a single administration of IFN without subsequent IFN administration. As a result, it was possible to observe the differences in HCV kinetics after a single administration between HCV genotypes and even between HCV subtypes.

PATIENTS AND METHODS

Patients

A total of 208 patients infected with HCV, with pretreatment HCV RNA concentration $>100 \times 10^3$ IU/ml as assessed by quantitative polymerase chain reaction (PCR) assay (Amplicor GT-HCV Monitor, Version 2.0; Roche Molecular Systems, Pleasanton, CA) were enrolled in the study. The clinical characteristics of the study patients are listed in Table I. These patients had been candidate for the antiviral therapy with PEG-IFN and ribavirin and had started the therapy between January 2005 and August 2007. They comprised 94 (45.2%) males and 114 (54.8%) females, with a mean age of 57.8 ± 9.9 years. Pretreatment HCV RNA concentration was $1{,}948 \pm 1{,}897 \times 10^3$ IU/ml. Eighty-four patients (40.4%) had a history of previous antiviral therapy (retreatment case). Out of 194 patients who underwent pretreatment liver biopsy, the grade of liver fibrosis was F0 in 9 (4.6%) patients, F1 in 128 (66.0%) patients, F2 in 40 (20.6%) patients, and F3 in 17 (8.8%) patients, according to the METAVIR score [The French METAVIR Cooperative Study Group, 1994].

HCV genotype was measured by amplification of core-gene sequences with PCR using genotype-specific primers [Ohno et al., 1997]. HCV genotype was 1B in 132 patients (63.5%), 2A in 58 patients (27.9%), and 2B in 18 patients (8.6%).

The study protocol was approved by the institutional review board and was carried out in compliance with the Helsinki declaration. Written informed consent was obtained from each patient before enrollment in the study.

Single Administration of Standard Interferon-Alpha and Measurement of Serum HCV RNA Concentration

All patients underwent single administration of standard IFN-alpha (Fig. 1). They received an intramuscular injection of 6 megaunits of standard IFN-alpha-2b (Intron A; Schering-Plough, Tokyo, Japan). HCV RNA concentration was measured before and 24, 48, 72, and 120 hr after the injection of IFN-alpha. The changes in HCV RNA concentration were calculated in comparison with HCV RNA concentration before the injection.

TABLE I. Clinical Characteristics of Study Patients (n = 208)

Age (years)	57.8 ± 9.9
Sex (female/male)	114 (54.8)/94 (45.2)
History of interferon therapy (naive/retreatment)	124 (59.6)/84 (40.4)
Alanine aminotransferase (IU/L)	55.8 ± 56.3
Aspartate aminotransferase (IU/L)	48.4 ± 41.7
Gamma-glutamyl transpeptidase (IU)	48.8 ± 62.2
Alkaline phosphatase (IU/L)	266.7 ± 108.1
Albumin (g/dl)	4.12 ± 0.40
Total bilirubin (mg/dl)	0.66 ± 0.25
White blood cell count (/μl)	$5{,}047 \pm 1{,}359$
Hemoglobin (g/dl)	14.0 ± 1.4
Platelet count ($\times 10^3$/μl)	17.3 ± 5.6
Liver histology-activity (A0/A1/A2/A3)[a]	3 (1.5)/122 (62.9)/56 (28.9)/13 (6.7)
Liver histology-fibrosis (F0/F1/F2/F3)[a]	9 (4.6)/128 (66.0)/40 (20.6)/17 (8.8)
HCV genotype (1B/2A/2B)	132 (63.5)/58 (27.9)/18 (8.6)
HCV RNA concentration ($\times 10^3$ IU/ml)	$1{,}948 \pm 1{,}897$
Response (SVR/relapse/NR)[b]	103 (51.5)/63 (31.5)/34 (17.0)

HCV, hepatitis C virus; SVR, sustained virologic response; NR, no response.
Percentages are shown in parentheses.
[a]Liver biopsy was not performed in 14 patients.
[b]Eight patients dropped out.

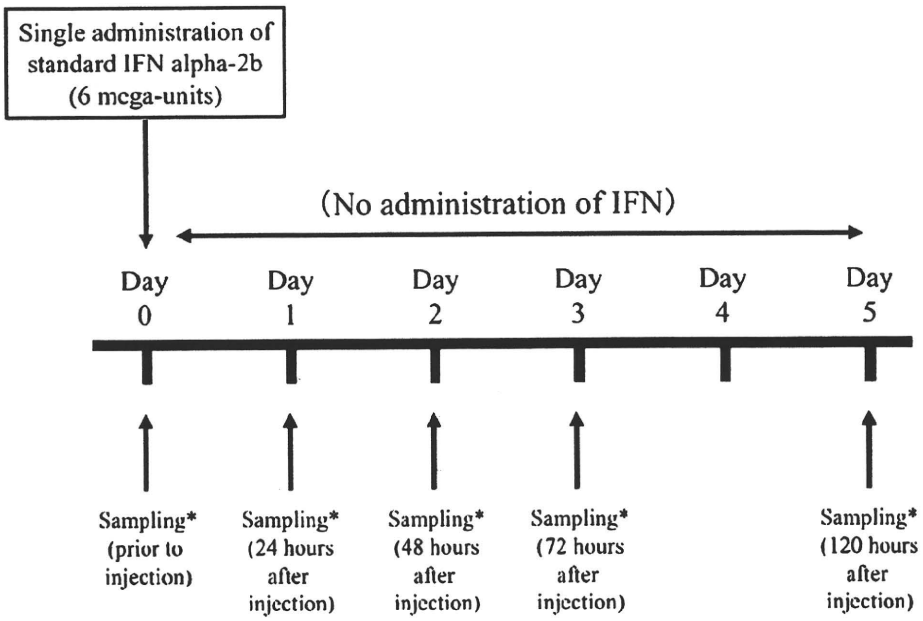

Fig. 1. Schematic representation of a single administration of standard interferon-alpha and measurements of HCV RNA concentration. Serum HCV RNA concentration was measured before, and 24, 48, 72, and 120 hr after a singe administration of 6 megaunits of standard interferon-alpha. Antiviral therapy with peginterferon and ribavirin was started at least 2 weeks after the examination. IFN, interferon; sampling*, sampling of serum samples for the measurement of HCV RNA concentration.

Antiviral Combination Therapy With Peginterferon and Ribavirin

All patients underwent antiviral combination therapy with PEG-IFN and ribavirin more than 2 weeks after the single administration of standard IFN. The initial doses of PEG-IFN and ribavirin, and the reduction in their doses, were according to the manufacturer's recommendations. Patients with HCV genotype 1B underwent a 48-week treatment regimen, whereas those with genotype 2A or 2B underwent a 24-week regimen. As for the outcome of the combination therapy, patients were classified as sustained virologic response when serum HCV RNA turned negative during treatment and remained so 6 months after the end of treatment (i.e., eradication of HCV). They were classified as relapse when serum HCV RNA turned negative during the treatment period but returned to be positive after the end of treatment. They were defined as no response, when serum HCV RNA remained positive throughout the treatment period and thereafter.

Statistical Analysis

Quantitative values are shown as mean ± SD. Between-group differences were analyzed by chi-square test. Differences in quantitative values between two groups were analyzed by the Mann–Whitney U-test. In comparison between more than two groups, Bonferroni's correction was performed. Multiple regression analysis was performed for factors that affected HCV kinetics after single administration of IFN. All P-values are two-tailed, and $P < 0.05$ was accepted as statistically significant.

RESULTS

Background Characteristics of Patients With HCV Genotypes 1B and 2A/2B and Responses to Antiviral Combination Therapy With Peginterferon and Ribavirin

Table II summarizes the background characteristics of patients infected with HCV genotype 1B and those infected with HCV genotype 2A or 2B. Serum alanine aminotransferase activity and aspartate aminotransferase activity were significantly higher in patients with HCV genotype 1B than those with HCV genotype 2A/2B, and pretreatment white blood cell counts tended to be higher in patients with genotype 1B. We found no difference in patient age, sex, history of previous IFN therapy, liver histology (both activity and fibrosis), or pretreatment HCV RNA concentration between genotypes.

As for the responses to the antiviral combination therapy with PEG-IFN and ribavirin, 52 of 129 patients with HCV genotype 1B (40.3%) achieved sustained virologic response, whereas 51 of 71 patients with HCV genotype 2A/2B achieved sustained virologic response. The rate of sustained virologic response was significantly higher in patients infected with HCV genotype 2A/2B (71.8%) than in those with genotype 1B ($P < 0.0001$). In contrast, the rate of no response was significantly higher in patients infected with HCV genotype 1B (24.8%) than in those with genotype 2A/2B (2.8%, $P < 0.0001$). Among patients infected with HCV genotype 2A/2B, the rate of sustained virologic response (SVR) was 39 of 54 (72.2%) in patients with

TABLE II. Baseline Characteristics of Patients With HCV Genotype 1B and Those With Genotype 2A/2B

	Genotype 1B (n = 132)	Genotype 2A/2B (n = 76)	*P*-value
Age (years)	58.5 + 8.7	56.4 ± 11.5	0.4218
Sex (female/male)	67 (50.8) ± 65 (49.2)	47 (61.8)/29 (38.2)	0.1609
History of interferon therapy (naive/retreatment)	78 (59.1)/54 (40.9)	46 (60.5)/30 (39.5)	0.9550
Alanine aminotransferase (IU/L)	59.5 ± 59.3	49.3 ± 50.4	0.0109
Aspartate aminotransferase (IU/L)	51.0 ± 40.0	44.0 ± 44.4	0.0224
Gamma-glutamyl transpeptidase (IU)	46.6 ± 46.6	52.6 ± 82.8	0.4877
Alkaline phosphatase (IU/L)	275.7 ± 114.0	251.0 ± 95.6	0.1560
Albumin (g/dl)	4.11 ± 0.41	4.13 + 0.38	0.7196
Total bilirubin (mg/dl)	0.67 ± 0.26	0.63 ± 0.24	0.4663
White blood cell count (/μl)	5,172 ± 1,327	4,830 ± 1,396	0.0646
Hemoglobin (g/dl)	13.9 ± 1.4	14.0 ± 1.4	0.7053
Platelet count ($\times 10^3$/μl)	17.0 ± 5.4	17.9 ± 5.7	0.3350
Liver histology-activity (A0/A1/A2/A3)[a]	2 (1.6)/73 (57.9)/41 (32.6)/10 (7.9)	1 (1.5)/49 (72.0)/15 (22.1)/3 (4.4)	0.2720
Liver histology-fibrosis (F0/F1/F2/F3)[a]	5 (4.0)/78 (61.9)/30 (23.8)/13 (10.3)	4 (5.9)/50 (73.5)/10 (14.7)/4 (5.9)	0.2593
HCV RNA concentration ($\times 10^3$ IU/ml)	1,841 ± 1,470	2,134 ± 2,471	0.8007
Response (SVR/relapse/NR)[b]	52 (40.3)/45 (34.9)/32 (24.8)	51 (71.8)/18 (25.4)/2 (2.8)	<0.0001

HCV, hepatitis C virus.
Percentages are shown in parentheses.
[a]Liver biopsy was not performed in 14 patients.
[b]Eight patients dropped out.

genotype 2A and 12 of 17 (70.6%) in those with genotype 2B; this rate was not significantly different between these two subgroups ($P = 0.8961$).

Differences in Decrease of Serum HCV RNA Concentration After Single Administration of Standard Interferon According to HCV Genotype

Figure 2 shows the changes in serum HCV RNA concentration after a single administration of standard IFN in patients with HCV genotypes 1B and 2A/2B. The reduction of serum HCV RNA concentration at 24 hr after the single administration was more marked in patients with HCV genotypes 2A/2B, and this difference

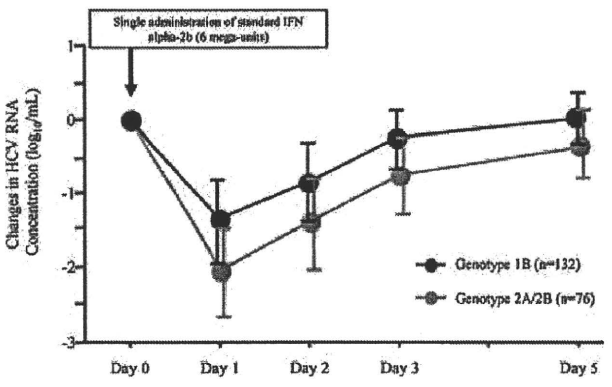

Fig. 2. Changes in HCV RNA concentration after a single administration of standard interferon between genotypes 1B and 2A/2B. The decrease for genotypes 1B and 2A/2B was $1.39 \pm 0.57 \log_{10}$ IU/ml versus $2.09 \pm 0.58 \log_{10}$ IU/ml at 24 hr, $0.85 \pm 0.53 \log_{10}$ IU/ml versus $1.42 \pm 0.61 \log_{10}$ IU/ml at 48 hr, $0.25 \pm 0.41 \log_{10}$ IU/ml versus $0.76 \pm 0.52 \log_{10}$ IU/ml at 72 hr, and $0.05 \pm 0.35 \log_{10}$ IU/ml versus $0.46 \pm 1.21 \log_{10}$ IU/ml at 120 hr after a single administration, all $P < 0.0001$.

was maintained at 48, 72, and 120 hr. The differences were significant at all points of measurement ($P < 0.0001$, Fig. 3). No difference was found in the HCV RNA concentration between before single administration of IFN and at 120 hr after single administration in both patients with genotype 1B and those with genotype 2A/2B; HCV RNA concentration restored to pre-administration level at 120 hr. The changes in HCV RNA concentration did not differ by age, body weight, or pretreatment HCV RNA concentration. The decrease in HCV RNA concentration was more marked in female patients than male patients ($1.78 \pm 0.67 \log_{10}$ IU/ml vs. $1.48 \pm 0.63 \log_{10}$ IU/ml, $P = 0.0014$).

The factors that associated with the decrease in HCV RNA concentration at 24 hr after single IFN administration were analyzed. In univariate analysis, patient sex ($P = 0.0011$), serum ALT activity ($P = 0.0464$), and HCV genotype ($P < 0.0001$) were significantly associated with the decrease, and serum AST activity ($P = 0.0795$) and histologic activity of hepatitis ($P = 0.0953$) were weakly associated with the decrease. In multivariate analysis, HCV genotype ($P < 0.0001$) and sex ($P = 0.0052$) affected independently the decrease of HCV RNA concentration at 24 hr after single administration (Table III). In the analysis of factors that associated with the decrease of HCV RNA concentration at 48 hr after single administration, sex ($P = 0.0265$) and HCV genotype ($P < 0.0001$) were significantly associated with the decrease, and serum ALT activity ($P = 0.0732$) and serum AST activity ($P = 0.0870$) were weakly associated with the decrease in univariate analysis. In multivariate analysis, only HCV genotype affected independently the decrease of HCV RNA concentration at 48 hr after single administration ($P < 0.0001$, Table IV).

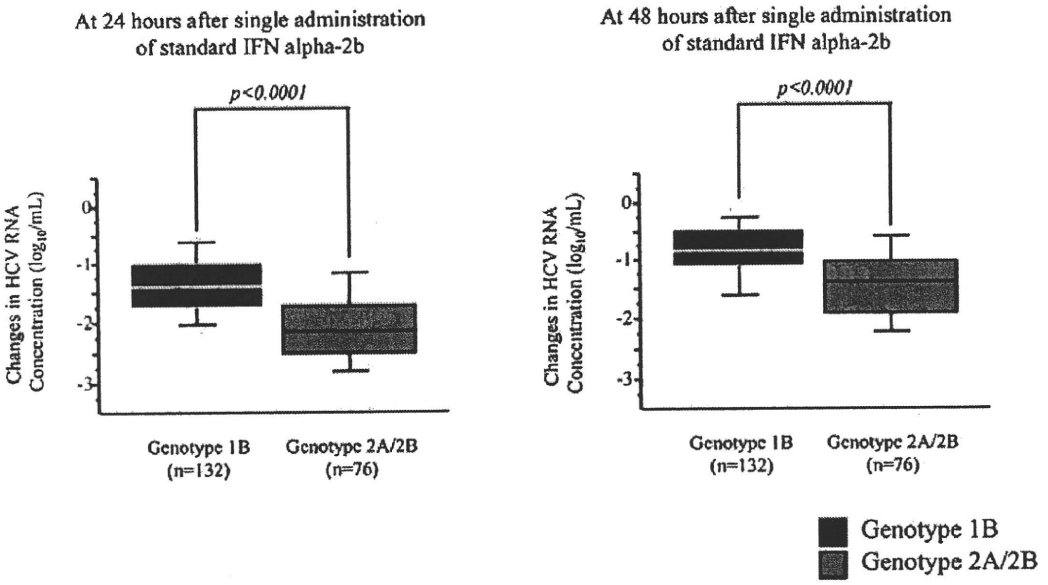

Fig. 3. Comparisons of the decrease in HCV RNA concentration at 24 and 48 hr after a single administration of standard interferon between genotypes 1B and 2A/2B. The decrease for genotypes 1B and 2A/2B was $1.39 \pm 0.57 \log_{10}$ IU/ml versus $2.09 \pm 0.58 \log_{10}$ IU/ml, $P < 0.0001$ at 24 hr, and $0.85 \pm 0.53 \log_{10}$ IU/ml versus $1.42 \pm 0.61 \log_{10}$ IU/ml, $P < 0.0001$ at 48 hr after a single administration.

Differences in Increase of Serum HCV RNA Concentration After Reduction by Single Administration of Standard Interferon According to HCV Genotype

Relative to its level at 24 hr after a single administration of standard IFN, HCV RNA concentration increased at 48, 72, and 120 hr after administration (Fig. 2). When HCV RNA concentration was compared for different genotypes between 24 and 48 hr, and between 24 and 72 hr, the increase was significantly more marked in patients with HCV genotype 2A/2B for both comparisons (genotype 1B vs. genotype 2A/2B; $0.53 \pm 0.36 \log_{10}$ IU/ml vs. $0.67 \pm 0.39 \log_{10}$ IU/ml, $P = 0.0220$ for 24–48 hr, and $1.14 \pm 0.44 \log_{10}$ IU/ml vs. $1.33 \pm 0.51 \log_{10}$ IU/ml, $P = 0.0024$ for 24–72 hr). When 34 patients with the decrease in HCV RNA concentration at 24 hr of $< 1.0 \log_{10}$ IU/ml were excluded, the increases in HCV RNA concentration during 24–48 and 24–72 hr after single administration were not different between genotypes ($P = 0.2248$ for 24–48 hr and $P = 0.0668$ for 24–72 hr, respectively, Fig. 4).

Differences Between HCV Genotypes 2A and 2B in Changes in Serum HCV RNA Concentration After Single Administration of Standard Interferon

The changes in HCV RNA concentration after a single administration of IFN were compared between patients with genotype 2A (n = 58) and genotype 2B (n = 18) (Figs. 5 and 6). The decrease in HCV RNA concentration was significantly more marked in patients with HCV genotype 2A than in those with genotype 2B at all points of measurement ($P = 0.0009$ at 24 hr, $P = 0.0005$ at 48 hr, $P = 0.0123$ at 72 hr, and $P = 0.0401$ at 120 hr). In contrast, the difference in HCV RNA concentration was not significant between patients with HCV genotype 1B and those with genotype 2B at 48 and 120 hr after single administration (Figs. 5 and 6).

DISCUSSION

A number of clinical studies have demonstrated that the rate of sustained virologic response (i.e., the rate of

TABLE III. Multiple Regression Analysis for Factors That Potentially Affected the Decrease of HCV RNA Concentration at 24 hr After the Injection of Standard Interferon-Alpha

Factor	Parameter estimate	Standard error	t-Value	P-value
Sex	0.1169	0.0413	2.83	0.0052
Alanine aminotransferase	−0.0010	0.0018	−0.57	0.5705
Aspartate aminotransferase	0.0004	0.0025	0.18	0.8550
Activity of hepatitis	−0.0373	0.0431	−0.87	0.3871
Genotype	0.3350	0.0433	7.74	<0.0001

TABLE IV. Multiple Regression Analysis for Factors That Potentially Affected the Decrease of HCV RNA Concentration at 48 hr After the Injection of Standard Interferon-Alpha

Factor	Parameter estimate	Standard error	*t*-Value	*P*-value
Sex	0.0610	0.0394	1.55	0.1226
Alanine aminotransferase	−0.0006	0.0017	−0.37	0.7112
Aspartate aminotransferase	−0.0003	0.0023	−0.11	0.9145
Genotype	0.2756	0.0404	6.81	<0.0001

HCV eradication) with antiviral therapy with IFN/PEG-IFN or with the combination of IFN/PEG-IFN and ribavirin is greater in patients infected with HCV genotype 2, compared with patients infected with HCV genotype 1 [Yoshioka et al., 1992; McHutchison et al., 1998; Poynard et al., 1998; Zeuzem et al., 2000; Lindsay et al., 2001; Manns et al., 2001; Fried et al., 2002; Hatziyannis et al., 2004]. However, the mechanism of this favorable response of patients infected with HCV genotype 2 to the antiviral therapy has not been fully elucidated.

The importance of HCV kinetics associated with these antiviral therapies has been reported in the prediction of the response to the therapy. Initial viral dynamic studies showed that IFN therapy produces a biphasic decline in HCV RNA concentration after a certain delay [Lam et al., 1997; Bekkering et al., 1998; Neumann et al., 1998; Layden-Almer and Layden, 2003]. The changes in HCV RNA concentration that occur over the first 24 hr after the injection of IFN are considered to be the first phase, during which there is a rapid decrease in serum HCV RNA concentration of reportedly 0.5–2.0 $\log_{10}$ IU/ml. The decline in HCV RNA concentration during the first phase is reportedly determined by the effectiveness of IFN at inhibiting viral replication and the clearance of circulating virus [Layden-Almer et al., 2006]. It does not reflect the clearance of HCV-infected cells but reflects purely the effect on the circulating HCV. The half-life of HCV has been predicted to be 2.7 hr and the daily production rate of HCV has been estimated to be 10^{12} virions [Neumann et al., 1998]. IFN that can inhibit this replication of HCV produces a reduction in HCV RNA concentration.

In the present study, changes in HCV RNA concentration were examined at 24, 48, 72, and 120 hr after a single administration of standard IFN. Because we did not repeat the administration of IFN after the initial administration, the restoration of HCV RNA concentration was observed at 48, 72, and 120 hr after a single administration in comparison with the concentration at 24 hr. The dose of standard IFN was fixed at 6 megaunits because we usually administered this dose previously, during the period when monotherapy with standard IFN was the standard therapy for patients with chronic hepatitis C [Toyoda et al., 1996, 1997]. Significant difference in HCV RNA concentrations was found at

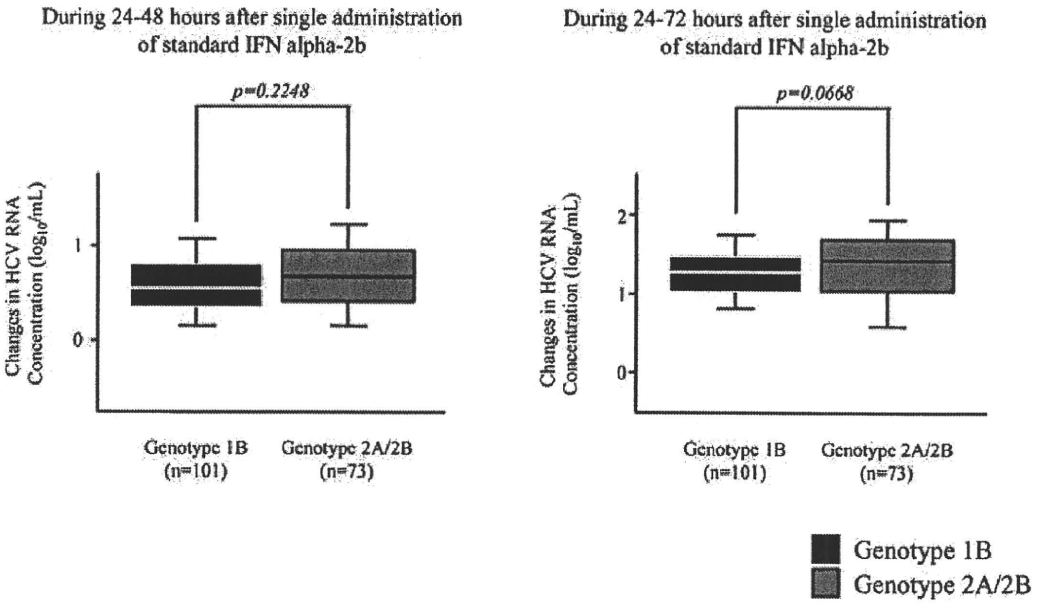

Fig. 4. Comparisons of the increase in HCV RNA concentration during 24–48 and 24–72 hr after a single administration of standard interferon between genotypes 1B and 2A/2B. The increase for genotypes 1B and 2A/2B was 0.60 ± 0.38 $\log_{10}$ IU/ml versus 0.68 ± 0.39 $\log_{10}$ IU/ml, $P = 0.2248$ during 24–48 hr, and 1.26 ± 0.40 $\log_{10}$ IU/ml versus 1.36 ± 0.50 $\log_{10}$ IU/ml, $P = 0.0668$ during 24–72 hr.

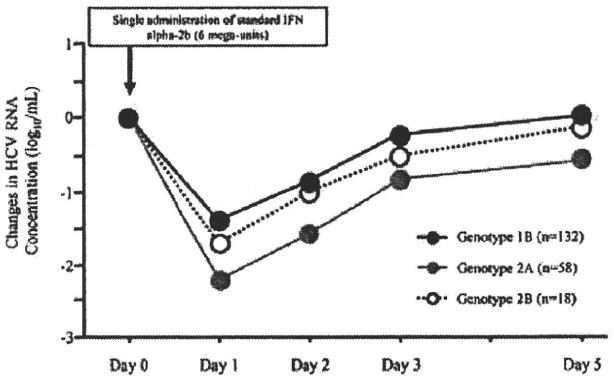

Fig. 5. Changes in HCV RNA concentration after a single administration of standard interferon between genotypes 2A and 2B. The decrease for genotypes 2A and 2B was $2.21 \pm 0.53 \log_{10}$ IU/ml versus $1.68 \pm 0.56 \log_{10}$ IU/ml, $P = 0.0009$, at 24 hr, $1.55 \pm 0.60 \log_{10}$ IU/ml versus $1.00 \pm 0.44 \log_{10}$ IU/ml, $P = 0.0005$, at 48 hr, $0.83 \pm 0.53 \log_{10}$ IU/ml versus $0.51 \pm 0.36 \log_{10}$ IU/ml, $P = 0.0123$, at 72 hr, and $0.55 \pm 1.35 \log_{10}$ IU/ml versus $0.15 \pm 0.40 \log_{10}$ IU/ml, $P = 0.0401$, at 120 hr after single administration. For comparison between genotypes 1B and 2B, the P-values were 0.0412, 0.2213, 0.0076, and 0.3935, respectively, at 24, 48, 72, and 120 hr after a single administration.

24, 48, 72, and 120 hr after the single administration of standard IFN between patients infected with HCV genotype 1B and those with HCV genotype 2A/2B. The reduction in HCV RNA concentration was significantly greater in patients with HCV genotype 2A/2B than in those with genotype 1B, and this difference was maintained at 48, 72, and 120 hr. The difference in the rate of sustained virologic response between patients with genotype 1B and those with 2A/2B could be accounted for by this difference in HCV dynamics after a single administration of IFN.

The reason for this difference in HCV kinetics after a single administration of IFN is not known. The antiviral effect of IFN is reportedly dose-dependent; therefore, the difference in body weight of patients might influence the effect of a single administration of IFN, since the dose was fixed at 6 megaunits for all patients. However, we found no difference in body weight between patients with genotype 1B and those with genotype 2A/2B, and, in addition, we did not find differences in the changes of serum HCV RNA concentration according to the body weight of patients. HCV genotype 2 is apparently more sensitive to IFN as an antiviral agent than HCV genotype 1 is, and this difference in the sensitivity to IFN between genotypes might have resulted in the difference in HCV kinetics after single IFN administration.

The faster reduction of genotype 2 HCV RNA at 24 hr after single administration (i.e., during the first phase) could be due not just to a difference in sensitivity to IFN but also to a faster free virion clearance rate. Several studies [Yoshioka et al., 1997; Hattori et al., 1998; Mondelli et al., 1999] have shown that antibody responses to the hypervariable region 1 (HVR-1) of the HCV envelope glycoprotein E2 were significantly more vigorous and frequent in HCV genotype 2-infected subjects, in comparison to genotype 1 patients. Furthermore, the more rapid variation in HVR-1 viral sequences from genotype 2 patients, in comparison with genotype 1 [Brambilla et al., 1998], also suggests a

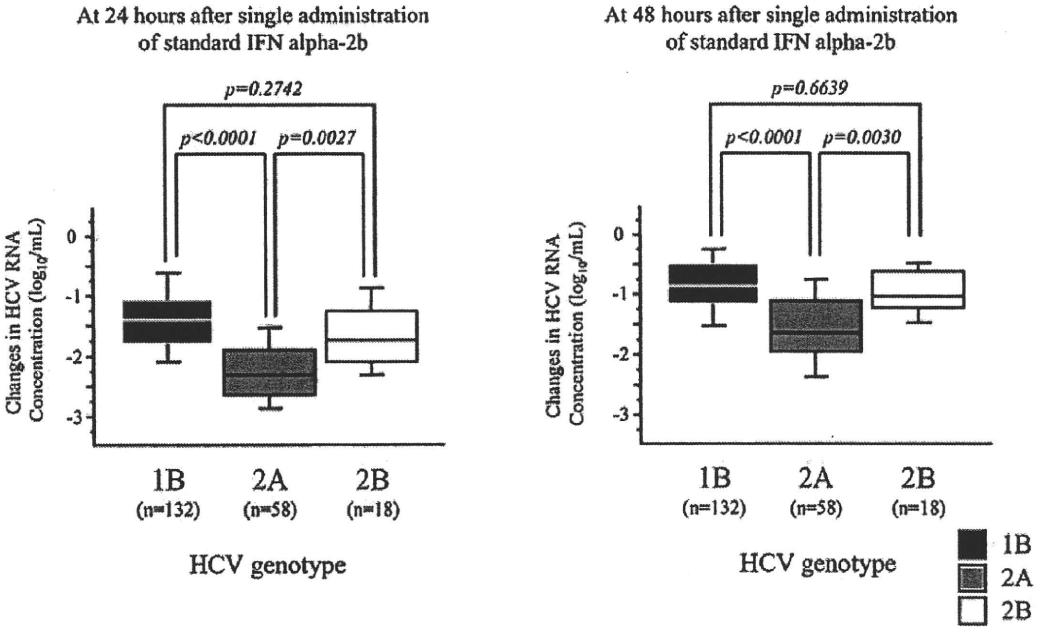

Fig. 6. Comparisons of the decrease in HCV RNA concentration at 24 and 48 hr after a single administration of standard interferon between genotypes 2A and 2B. The decrease for genotypes 2A and 2B was $2.21 \pm 0.53 \log_{10}$ IU/ml versus $1.68 \pm 0.56 \log_{10}$ IU/ml, $P = 0.0009$ at 24 hr, and $1.55 \pm 0.60 \log_{10}$ IU/ml versus $1.00 \pm 0.44 \log_{10}$ IU/ml, $P = 0.0005$ at 48 hr after a single administration.

stronger antibody immune pressure on genotype 2 virus. These observed differences in antibody responses may explain, in part, the differences in HCV kinetics between genotypes after a single administration of IFN.

Another hypothesis for the better response to treatment in patients infected with HCV genotype 2 is that the restoration of HCV RNA concentration following the decrease by an administration of IFN of this genotype is slower than that of genotype 1. However, in the present study, the increase in HCV RNA concentration following the decrease at 24 hr by a single IFN administration was not greater in patients with HCV genotype 1; rather, the increase in HCV RNA concentration from 24 to 48 hr or from 24 to 72 hr was greater in patients with HCV genotype 2. We reanalyzed the increase of HCV RNA concentration during these periods excluding patients in whom HCV RNA concentration did not markedly decrease by a single administration and the reduction at 24 hr was <1.0 $\log_{10}$ IU/ml, because the increase of HCV RNA concentration would be expected to be smaller if the initial decrease in HCV RNA concentration (at 24 hr) was small. Even with this exclusion, the increase in HCV RNA concentration in patients with HCV genotype 2 was not smaller than in those with genotype 1. These results show that the restoration of HCV genotype 2 is not slower than that of genotype 1, and that this would not contribute to the favorable response to IFN in patients with HCV genotype 2. Neumann et al. [1998] reported that there was no evidence for a difference in replication of HCV between genotypes, and suggested the necessity for a large study with frequent measurements of HCV kinetics during treatment or after its cessation to illuminate possible differences in replication rates between the genotypes. Our results might have added some information on this issue.

Interestingly, significant differences were found in HCV RNA concentration after single administration of IFN between patients with HCV genotypes 2A and 2B. The decrease in HCV RNA concentration at 24 hr after single administration in patients with HCV genotype 2B was smaller than that in patients with genotype 2A, and the changes in HCV RNA in patients with HCV genotype 2B were more similar to that in patients with genotype 1B than to that in patients with genotype 2A. This is surprising in light of our result that the rate of sustained virologic response of patients with genotype 2B (70.6%) was comparable to that of patients with genotype 2A (72.2%) and was significantly higher than that of patients with genotype 1B (40.3%). The previous study by Karino et al. [2003] reported the lack of difference in the decrease of HCV RNA concentration at 24 hr after administration of IFN between patients with HCV genotypes 2A and 2B. This discrepancy might be partly because the number of patients with HCV genotype 2B was small in both studies ($n = 5$ in the study by Karino et al. and $n = 18$ in the present study). An increase in the sample size of patients with genotype 2B will clarify whether there is a difference in HCV kinetics between HCV genotypes 2A and 2B and whether different factors may play a role in the comparable high rate of SVR between patients with HCV genotype 2A and those with HCV genotype 2B. Further studies will be needed to elucidate this issue.

In conclusion, a significant difference was found in HCV kinetics after a single administration of standard IFN between genotypes (genotypes 1B vs. 2A/2B), and between subtypes (genotype 2A vs. 2B). The difference in HCV kinetics between genotypes 1B and 2A/2B appeared to be due mainly to the difference in the reduction of HCV RNA concentration after the administration of IFN and not due to the difference in restoration of HCV RNA concentration. The difference in HCV kinetics between genotypes 1B and 2A/2B observed in the present study may be associated with the difference in the rate of SVR to the combination therapy of PEG-IFN and ribavirin. In contrast, HCV kinetics after the administration of IFN were also different between HCV genotypes 2A and 2B, despite their similar rate of SVR to the combination therapy with PEG-IFN and ribavirin. Further studies will be needed to compare the HCV kinetics to IFN, including patients with HCV genotype 1A or genotype 3, and to investigate the mechanism of the observed differences in HCV kinetics between genotypes.

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Transarterial chemotherapy alone versus transarterial chemoembolization for hepatocellular carcinoma: A randomized phase III trial☆

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***Background/Aims*: Transcatheter arterial chemoembolization (TACE) is a combination of transarterial infusion chemotherapy (TAI) and embolization, and has been widely used to treat patients with hepatocellular carcinoma (HCC). However, since the impact of adding embolization on the survival of patients treated with TAI had never been evaluated in a phase III study, we conducted a multi-center, open-label trial comparing TACE and TAI to assess the effect of adding embolization on survival.**

***Methods*: Patients with newly diagnosed unresectable HCC were randomly assigned to either a TACE group or a TAI group. Zinostatin stimalamer was injected into the hepatic artery, together with gelatin sponge in the TACE group and without gelatin sponge in the TAI group. Treatment was repeated when follow-up computed tomography showed the appearance of new lesions in the liver or re-growth of previously treated tumors.**

***Results*: Seventy-nine patients were assigned to the TACE group, and 82 were assigned to the TAI group. The two groups were comparable with respect to their baseline characteristics. At the time of the analysis, 51 patients in the TACE group and 58 in the TAI group had died. The median overall survival time was 646 days in the TACE group and 679 days in the TAI group ($p = 0.383$).**

***Conclusions*: The results of this study suggest that treatment intensification by adding embolization did not increase survival over TAI with zinostatin stimalamer alone in patients with HCC.**

Keywords: **Zinostatin stimalamer; Survival benefit; Overall survival; Lipiodol emulsion; Gelatin sponge**

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Abbreviations: HCC, hepatocellular carcinoma; AFP, α-fetoprotein; TACE, transarterial chemoembolization; TAI, transarterial infusion chemotherapy; SMANCS, zinostatin stimalamer; CT, computed tomography; TE, therapeutic effect; SMA, styrene maleic acid; NCS, neocarzinostatin.

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and a major cause of cancer mortality [1]. Although the screening of populations with a high risk of HCC using ultrasonography and serum α-fetoprotein (AFP) measurements have recently increased the number of candidates for effective local treatments such as hepatic resection and local ablation therapy, many patients exhibit HCCs that are unsuitable for local treatments at the time of the initial diagnosis or at the time of recurrence after local treatment. In these patients, transcatheter arterial chemoembolization (TACE) has been widely used, because TACE induces a marked antitumor effect in HCC.

Several randomized controlled studies have been conducted to assess the survival benefit of TACE compared with conservative therapy [2–9], and an improvement in survival with TACE has been shown in two recent phase III studies [7,8], in both of which TACE was compared with no treatment, and in two meta-analyses [10,11]. However, the impact of adding embolization on the overall survival of patients treated with transarterial infusion chemotherapy (TAI) has never been evaluated in a randomized controlled phase III study. We conducted a multi-centre, open-label trial to compare the effects of TACE and TAI alone to clarify the possible benefits of treatment intensification using embolization in addition to infusion chemotherapy. In this study, zinostatin stimalamer (SMANCS) was selected as the chemotherapeutic agent for use with both TACE and TAI. SMANCS is a lipophilic anti-cancer agent that dissolves in lipiodol to form a stable solution, retaining selectively in HCC. TAI with SMANCS has been widely used in clinical practice to treat patients with advanced HCC in Japan, because it has been reported to have fewer deleterious effects than TACE, especially on liver function, and to have an antitumor effect superior to TAI with other water-soluble agents in non-randomized studies [12,13].

2. Methods

Consecutive new patients with HCC were eligible if they had no indications for resection and/or local ablation therapy. The diagnosis was confirmed histologically and/or clinically using angiography and computed tomography (CT). Each patient was required to meet the following criteria: intrahepatic lesions that showed tumor staining by angiography and those in which the total size was less than 50% of the entire liver; adequate hematological function (white blood cells $\geqslant 3000/mm^3$, platelets $\geqslant 50{,}000/mm^3$, and hemoglobin $\geqslant 9.0$ g/dL), adequate hepatic function (serum total bilirubin $\leqslant 2.0$ mg/dL, serum albumin $\geqslant 3.0$ g/dL, serum AST [aspartate aminotransferase] $\leqslant 5$ times the upper limit of normal, serum ALT [alanine aminotransferase] $\leqslant 5$ times the upper limit of normal); adequate renal function (serum creatinine < the upper limit of normal, and serum blood urea nitrogen < the upper limit of normal); an Eastern Cooperative Oncology Group performance status of 0–1; an age of between 20 and 74 years of age; technically eligible for intra-arterial therapy; and written informed consent. Patients were excluded if they met any of the following criteria: a history of allergy to iodine-containing agents and/or contrast material; concomitant malignancy; a history of anti-cancer treatment for HCC; extrahepatic metastasis or tumor thrombus in the portal vein and/or the hepatic vein; intrahepatic arteriovenous shunting; ascites and/or pleural effusion not controlled by diuretics; pregnant or lactating woman and fertile patients not using effective contraception; myocardial infarction within the previous 6 months; or any serious physical and/or mental conditions. The study was performed in accordance with the Declaration of Helsinki, and approved by the ethics committee of each participating center. The study was investigator-designed and investigator-driven, and it received no support from any pharmaceutical companies.

Patients who met the eligibility criteria were provisionally registered before undergoing angiography. After confirmation of technical eligibility and reconfirmation of indications for the protocol intra-arterial treatments in regard to tumor status, including the number of tumors, their vascularity, and vascular invasion based on the angiographic findings, confirmatory registration was completed by each participating investigator. Central randomization to either a TACE group or TAI group was performed by using a telephone randomization system with stratification according to AFP level and treatment center. First, participants were stratified according to AFP level into a group with levels less than 400 ng/mL and a group with levels of 400 ng/mL or more. The group with AFP levels less than 400 ng/mL was further stratified according to treatment center. Randomization was achieved using a computer-generated allocation by permutation of blocks in equal proportions.

The treatments were performed by the participating investigators at 10 Japanese centers. Zinostatin stimalamer (SMANCS; Astellas Pharm Inc., Tokyo, Japan)/lipiodol emulsion (1 mg/mL) was injected slowly under fluoroscopic monitoring into the artery feeding the HCC using a catheter in a superselective manner in both the TACE and the TAI groups. The emulsion was prepared by suspending the SMANCS in lipiodol and shaking just before injection. The volume of the emulsion, up to a maximum of 6 mL (containing 6 mg of SMANCS), was adjusted according to the tumor size and tumor distribution. In the patients in the TACE group, gelatin sponge particles were utilized after the injection of the SMANCS-lipiodol emulsion. Treatment was repeated when a follow-up CT examination showed new lesions in the liver or re-growth of previously treated tumors. Treatment was discontinued if the size of the tumor treated had increased by more than 25% one month after the previous treatment; if there were any vascular contraindications, any exclusion criteria, or any severe adverse effects (defined as grade 4 leucopenia, grade 4 neutropenia, or grade 3 febrile leucopenia/neutropenia, a serum total bilirubin elevation of more than or equal to 5.0 mg/dL, a serum creatinine elevation of more than or equal to 1.5 times the upper normal limit, or grade 3 or greater non-hematological toxicity excluding nausea, vomiting, anorexia, pain, fever, hyperglycemia, fatigue, and serum transaminase elevation), or if the patient so requested.

The primary outcome measure was survival calculated from the date of randomization. Secondary outcome measures were tumor response and toxicity. Antitumor effect was evaluated by CT performed 1 month after the completion of treatment and every 3–4 months thereafter according to the response evaluation criteria proposed by the panel of experts of the Liver Cancer Study Group of Japan [14], which resemble the criteria proposed by the European Association for the Study of the Liver (EASL) Panel of Experts on HCC [15]. Tumor size was measured using the sum of the products of the perpendicular longest diameters of all measurable lesions. In the response evaluation criteria, lipiodol accumulation in the tumors is regarded as an indication of necrosis because significant positive correlations have been reported between lipiodol accumulation observed on CT images and the necrotic regions in resected tumors examined pathologically after TACE and after TAI with SMANCS [13,16,17]. Therapeutic effect V (TE V) is defined as the disappearance or 100% necrosis of all tumors, TE IV as a more than a 50% reduction in tumor size and/or more than 50% necrosis, and TE III as a more than 25% reduction and/or more than 25% necrosis. TE I is defined as a more than 25% increase in tumor size. TE II is defined as disease not qualifying for classification as TE V, IV, III, or I. The serum AFP level of each patient was also measured 1 month after treatment and every 3–4 months thereafter. Toxicity was assessed according to the criteria of the Japan Society for Cancer

Therapy [18], whose criteria are essentially the same as the WHO criteria [19]. The follow-up period was defined in the protocol as 2 years after the enrollment of the last patient.

2.1. Statistical analysis

Based on our previous phase II studies, in which we reported a 2-year survival rate of 80% in patients treated with TACE and of 60% in patients treated with TAI, 70 patients were required in each group to achieve a 90% power to detect superior survival in the TACE group by using a two-sided alpha level of 5% [13,20]. After sensitivity analyses of combinations of survival parameters, we targeted the recruitment of 80 patients in each group. All analyses were conducted based on the intention-to-treatment principle. Survival curves were calculated from the day of randomization using the Kaplan–Meier method and compared using the log-rank test. Comparisons between groups were made using the Wilcoxon test for continuous variables and Fisher's exact test for categorical variables. Analyses were conducted using SAS ver. 8.

3. Results

Between October 1999 and June 2003, 222 patients were provisionally enrolled in the study at 10 Japanese centers (Fig 1). Sixty-one of the 222 patients were excluded because of ineligibility for intra-arterial treatment based on the angiographic findings or withdrawal of consent; too few or too many definitive tumors that required reconsideration of the treatment strategy (46), tumor thrombus in the portal vein (3), tumors without sufficient tumor staining (3), intrahepatic arteriovenous shunting (2), allergy to contrast material (1), and withdrawal of consent (6). The most common reason for exclusion was having too few definitive tumors (37/61). The patients who were excluded because of having too few definitive tumors had been considered eligible based on the detection of several small hypervascular nodules on pre-treatment CT imaging that were diagnosed as HCC, but treatment had been switched to local ablation therapy or monitoring based on angiographic findings suggesting that the nodules represented dysplastic nodules. All of the patients who withdrew consent requested TACE for their treatments. The remaining 161 patients were allocated randomly to the TACE group (79 patients) or the TAI group (82 patients). Follow-up was continued through to June 17, 2005, two years after the enrollment of the last patient. Although the baseline data of some eligible patients did not meet the eligibility criteria after they were enrolled, the study protocol permitted initiation of treatment when according to the judgment of the investigator, treatment could be performed safely. Two patients had a pre-treatment serum albumin level that was below the eligibility criterion, but there were no statistically significant differences in baseline characteristics between the two groups (Table 1).

3.1. Treatment

The total number of treatment courses was 170 with a mean of 2.2 courses per patient (range, 1–9 courses) in the TACE group and 193 with a mean of 2.4 courses (range, 1–6 courses) in the TAI group. Eight patients in the TACE group and two patients in the TAI group were scheduled for the continuation of protocol treatment as of the date of the last follow-up. The remaining 71 patients in the TACE group and 80 patients in the TAI group had discontinued treatment. The reasons for treatment discontinuation were similar in both groups (Table 2).

3.2. Survival

At the time of the final analysis, 51 patients in the TACE group and 58 patients in the TAI group had died. Seven patients in the TACE group and eight in the TAI group were lost to follow-up after the cessation of protocol treatment. The median overall survival time was 646 days in the TACE group and 679 days in the TAI group. The estimated 2-year survival rate was 48.2% for the TACE group and 49.6% for the TAI group. No significant difference in survival was seen between the two groups ($p = 0.383$, Fig. 2).

3.3. Antitumor effect

The tumor response on CT was determined in 156 patients (77 in the TACE group and 79 in the TAI group). In the TACE group, there were 8 TE V, 29 TE IV, 31 TE III, 7 TE II, and 2 TE I responses. In the TAI group, there were 5 TE V, 22 TE IV, 30 TE III, 21 TE II, and 1 TE I response. The proportion of patients with TE V or IV among the measurable patients was not significantly different between the TACE group and the TAI group (48.1% vs. 34.2%; $p = 0.11$). There was no significant difference between the two groups in the proportion of patients with a pre-treatment AFP level > 200 ng/mL whose AFP level decreased by more than half (16.5% vs. 13.4%; $p = 0.66$).

3.4. Toxicity

Hematological toxicity was relatively mild and transient in both groups, although 2 patients (2.6%) in the TACE group and 3 (3.7%) in the TAI group developed grade 4 thrombocytopenia (Table 3). Major non-hematological toxicities were hyperbilirubinemia, elevations in serum liver enzymes, fever and abdominal pain in both groups. The grade of elevated ALT levels was significantly higher in the TACE group than in the TAI group, although there were no significant differences in any other toxicities between the two groups. No treatment-related death was observed in either group. Two patients in the TACE group and six in the TAI group manifested a grade 1–2 allergic reaction immediately after injection of the SMANCS-lipiodol emulsion. Shivering in the form of trembling of the whole body lasting

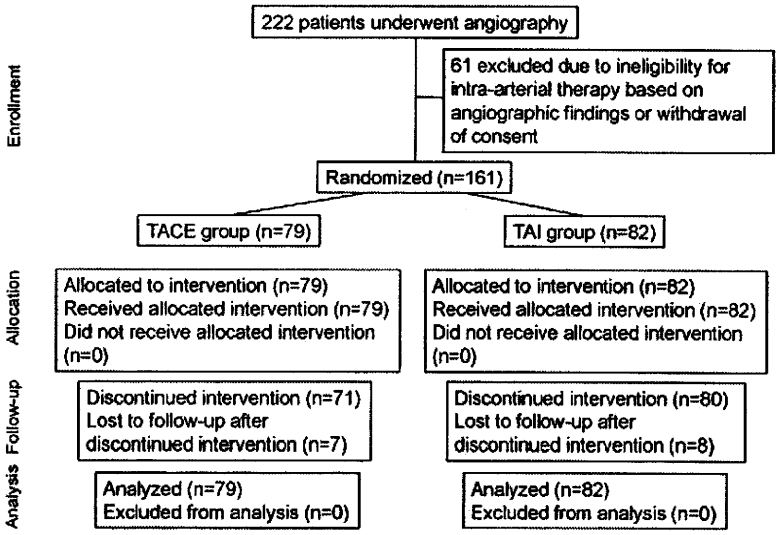

Fig. 1. Study flow diagram.

several minutes after the injection was noted in 12 patients in the TACE group and 14 patients in the TAI group, and it was thought to have been caused by SMANCS.

4. Discussion

We initiated this randomized study in 1999 because the impact of adding embolization on overall survival

Table 1
Baseline characteristics.

No. of patients		79	82
Age, year	Median (range)	65.0 (42–74)	67.0 (44–74)
Gender	Male	61 (77.2%)	70 (85.4%)
Performance status	0	76 (96.2%)	77 (93.9%)
	1	3 (3.8%)	5 (6.1%)
HBsAg	+	11 (13.9%)	7 (8.5%)
HCVAb	+	57 (72.2%)	60 (73.2%)
Alcohol abuse	+	33 (41.8%)	28 (34.1%)
Albumin, g/dL	Median (range)	3.6 (2.8–4.6)	3.6 (3.0–4.6)
Total bilirubin, mg/dL	Median (range)	1.0 (0.4–2.0)	0.9 (0.3–2.0)
AST, IU/L	Median (range)	63 (16–243)	69 (18–232)
ALT, IU/L	Median (range)	60 (12–184)	60 (10–213)
Prothrombin time, %	Median (range)	80 (41–129)	78.5 (43–111)
Platelet count, $\times 10^9$/L	Median (range)	110 (48–280)	120 (44–290)
	$<100 \times 10^9$/L	29 (36.7%)	28 (34.1%)
Ascites	+	3 (3.8%)	3 (3.7%)
Stage*	I	2 (2.5%)	4 (4.9%)
	II	18 (22.8%)	17 (20.7%)
	III	28 (35.4%)	25 (30.5%)
	IV-A	31 (39.2%)	36 (43.9%)
Tumor distribution	Unilateral	40 (50.6%)	36 (43.9%)
	Bilateral	39 (49.4%)	46 (56.1%)
Maximum tumor diameter, mm	Median (range)	35 (10–330)	35 (12–350)
Number or tumors	1	13 (16.5%)	11 (13.4%)
	2–5	43 (54.4%)	52 (63.4%)
	6	23 (29.1%)	19 (23.2%)
AFP, ng/ml	Median (range)	68.3 (2.8–79170)	93.8 (3.1–40,000)
	$\geqslant$400 ng/ml	26 (32.9%)	27 (32.9%)
Serum creatinine, mg/dL	Median (range)	0.7 (0.4–1.3)	0.8 (0.5–1.1)

Abbreviations: AFP, α-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibody.
Alcohol abuse was defined as ethanol intake $\geqslant$80 g/day for $\geqslant$5 years.
* According to the staging system of the Liver Cancer Study Group of Japan.

Table 2
Reasons for treatment discontinuation.

	TACE group		TAI group	
Ineffectiveness of protocol treatment	10	13%	10	12%
Adverse event caused by protocol treatment				
Elevation of serum creatinine level	1	1%	1	1%
Elevation of alkaline phosphatase level	2	3%	2	2%
Dyspnea	0	0%	1	1%
Hypotension	1	1%	1	1%
Shivers	0	0%	1	1%
Abdominal pain	0	0%	2	2%
Ascites	1	1%	0	0%
Deterioration before subsequent protocol treatment				
Extrahepatic metastasis	4	5%	7	9%
Portal vein thrombosis	6	8%	3	4%
Tumor rupture	2	3%	0	0%
Ascites	9	11%	11	13%
Liver dysfunction	9	11%	11	13%
Poor general condition	2	3%	2	2%
Other disease	1	1%	6	7%
Technical problem preventing subsequent protocol treatment	13	16%	9	11%
Patient's request	10	13%	11	13%
Indication for tumor ablation	1	1%	2	2%
Protocol treatment ongoing	7	9%	2	2%
Total	79		82	

for patients with advanced HCC treated with TAI had not been fully evaluated and because the efficacy of TACE was still being debated at that time in various countries. Moreover, several differences in TACE methods had been noted between clinical practice in East Asian countries, including Japan, and randomized studies conducted in Europe, including differences in the selection of embolization materials, anti-cancer agents and their doses, in treatment intervals, and in patient characteristics such as tumor stage and liver function. In this study, in which our TACE method was introduced, we selected SMANCS as a chemotherapeutic agent for both TACE and TAI. SMANCS is an anti-cancer drug that has been approved by the Japanese government for administration with lipiodol into the artery feeding HCC, and TAI with SMANCS has been widely used instead of TACE in many hospitals because of its favorable antitumor effect and mild toxicity profile.

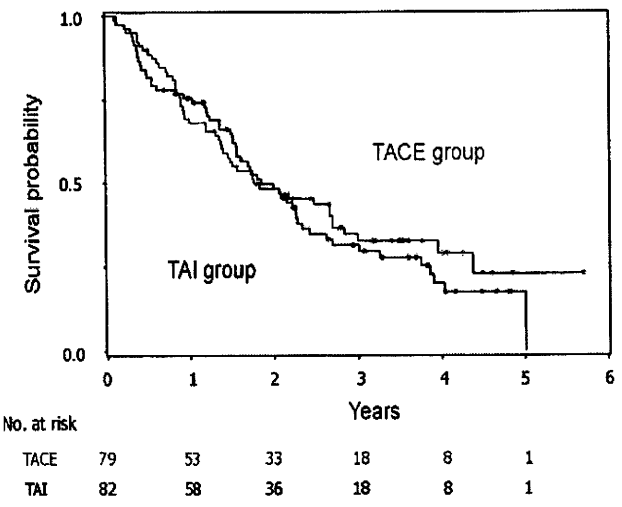

Fig. 2. Survival curves in the TACE group and in the TAI group.

This study did not confirm any significant survival advantage of TACE over TAI. A German group also reported that adding transient occlusion using degradable starch microspheres improved neither tumor response nor survival for patients treated with TAI using cisplatin and doxorubicin in a randomized phase II trial [21]. Llovet and Bruix showed that survival benefits were identified with TACE (doxorubicin or cisplatin) but not with embolization alone in their meta-analysis [11]. The survival benefit of TACE can be ascribed to the combination of embolization and chemotherapy.

It could be argued that the absence of a significant difference in survival rates between the TACE group and TAI group in this study is attributable to our methodological strategy for selecting SMANCS as the anti-cancer agent, because the agent may have produced favorable results in the TAI group. SMANCS is a high molecular weight chemical conjugate of a synthetic copolymer of styrene maleic acid (SMA) and the anti-cancer antibiotic protein, neocarzinostatin (NCS) [22,23]. SMANCS is lipophilic and dissolves in lipiodol to form a stable emulsion (SMANCS-lipiodol), which prevents rapid washout of SMANCS into plasma from trapped lipiodol. Furthermore, because of the enhanced permeability of the tumor vasculature and/or poor lym-

Table 3
Adverse events.

	TACE group						TAI group					
	Grade 3		Grade 4		Grade 1–4		Grade 3		Grade 4		Grade 1–4	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Hematological toxicity												
Leukocytes	1	1	0	0	27	34	0	0	0	0	26	32
Neutrophils	1	0	0	0	14	18	0	0	0	0	15	18
Hemoglobin	1	1	–		25	32	0	0	–		23	28
Platelets	10	13	2	3	54	68	10	12	3	4	57	70
Non-hematological toxicity												
Total bilirubin	21	27	0	0	60	76	15	18	0	0	62	76
Alkaline phosphatase	2	3	0	0	53	67	2	2	0	0	63	77
Aspartate aminotransferase	33	42	0	0	77	97	23	28	0	0	79	96
Alanine aminotransferase	28	35	0	0	73	92	16	20	0	0	77	94
Creatinine	0	0	0	0	13	16	0	0	0	0	16	20
Abdominal pain	0	0	0	0	55	70	2	2	0	0	50	61
Nausea/vomiting	1	1	–		43	54	0	0	–		39	48
Diarrhea	0	0	0	0	2	3	0	0	0	0	4	5
Fever	2	3	0	0	69	87	1	1	0	0	66	80
Shivers	0	0	0	0	12	15	1	1	0	0	14	17
Allergy	0	0	0	0	2	3	0	0	0	0	6	7
Ascites	1	1	–		3	4	0	0	–		0	0
Dyspnea	0	0	0	0	0	0	0	0	1	1	1	1
Hypotension	1	1	0	0	1	1	1	1	0	0	1	1

A 'dash' (–) indicates the grade was not available.

phatic drainage from the tumor interstitium, macromolecular agents like SMANCS are retained more selectively within tumors [24,25]. In fact, experimental studies have shown that tumor-systemic drug concentration ratios as high as 1000 can be achieved using TAI with SMANCS-lipiodol. Thus, the selective delivery of a long-lasting or slow-release anti-cancer agent may have had a sufficient antitumor effect and survival-prolonging efficacy in the TAI group even if embolization had not been used in combination.

The infrequent protocol treatment repetition in this study is another possible reason for the lack of any difference in survival between the two groups, because the average number of protocol treatments was only 2.2 courses in the TACE group and 2.4 in the TAI group, and thus the maximum anti-cancer potential may not have been achieved. We speculated that the choice of SMANCS was partly responsible for the infrequent repetition because hepatic vascular complications, such as the obstruction of the hepatic artery and the arterio-portal shunt, have been reported as adverse reactions specific to SMANCS [26]. These complications are often followed by liver dysfunction, ascites, and technical problems with regard to subsequent protocol treatment, which were the major reasons for treatment discontinuation in this study. The enrollment of many patients with far-advanced HCC in the present phase III study may have been another reason for the small number of treatment repetitions and the subsequent poor survival: the proportion of patients with a pre-treatment AFP level >200 ng/mL was 40% in the phase III study and 24% in the phase II study. Both the antitumor response and the overall survival of the TACE group were poorer than our expectations: the 2-year survival rate in the TACE group was 48.2% in the present study, as opposed to 79% in the phase II study of TACE with SMANCS.

In conclusion, the results of this study suggest that treatment intensification by adding embolization did not increase the survival of HCC patients over SMANCS transarterial chemotherapy alone. The results of this study also showed no significant differences in toxicity, except for an ALP elevation, between the two groups treated with SMANCS. It should be emphasized that the negative results in this study may be attributable to our methodological strategy for selecting SMANCS and the enrollment of many patients with far-advanced HCC. The infrequent treatment repetition and the favorable results of TAI with SMANCS are speculated to be reasons for the lack of any difference in survival between the two groups. Furthermore, the results of this study must be interpreted with caution because current TACE protocols have evolved thanks to the implementation of updated devices including new embolic agents and improved catheters. Additional studies will be required to determine whether the results obtained in this trial are consistent with the results of transarterial treatment with chemotherapeutic agents other than SMANCS and with updated procedures, although it would be difficult to conduct such studies because many consider TACE to be the standard treatment based on the positive results obtained in two recent randomized studies in which doxorubicin or cisplatin was used