Markov model to simulate the steps of stage progression of patients with small HCC under an intensive medical intervention. Here, we retrospectively evaluated the progression of HCC and the long-term prognosis of patients who had undergone RFA or surgical resection as the initial therapy for small HCCs, and assessed the prognostic factors of those patients.

The purposes of this study were, therefore, (i) to compare the recurrence rates, progression of tumour stage and survival rates between those patients who received percutaneous RFA and those who underwent surgery and (ii) to elucidate the significance of the selection of initial therapy for small HCCs from the viewpoints of stage progression and prognosis.

Patients and methods

Patients

A total of 468 patients were diagnosed as having a small HCC 3 cm or less in diameter, from March 1999 to April 2006, at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these 468 patients, 236 patients (50.4%) underwent percutaneous RFA therapy as a curative mode of treatment and the remaining 138 patients (29.5%) received surgical resection, 52 had TACE and the remaining 42 patients were treated with ethanol injection, microwave coagulation or other palliative methods of treatment.

A total of 374 consecutive patients with a small HCC, who underwent either RFA or surgery, were analysed in this study. None had been treated previously for HCC, and all had single or multinodular (up to three) HCCs 3 cm or less in diameter each, absence of portal venous thrombosis and known extrahepatic metastases, and Child–Pugh class A or B liver function.

The patients included 246 men and 128 women, and ranging in age from 29 to 87 years, with a median age of 65 years. The demography, laboratory data and features of cancer were compared between the two therapy groups (Table 1). Patients' age was lower in the surgery group by 4.5 years. The rate of HBV-positive disease was significantly higher in the surgery group, and liver function tests were also significantly better in the surgery group.

Hepatocellular carcinoma

Patients were required to have HCC with a definitive diagnosis by either typical hypervascular radiological features or histology through needle biopsy. Tumours had to be measurable by ultrasonography (US), computerized tomography (CT) and digital subtraction angiography. In order to elucidate the detailed characteristics of the HCC, CT during arterial portography and CT hepatic arteriography were performed in all the patients. Among 374 patients, HCC was confirmed by a resected specimen in 138 patients, by typical hypervascular characteristics on at least two modalities of imagings in 219 and by a fine-needle biopsy in 17.

Most patients (82.2%, 309 of 376) had a single tumour, and the median tumour diameter was 19 mm, ranging from 5 to 30 mm. The characteristics of the tumour in the subgroup of RFA and surgery are given in Table 1. The median size of the largest tumour was 18 mm in the RFA group and 20 mm in the surgery group ($P < 0.001$).

Table 1. Clinical features of the patients with small liver cancer

Initial therapy	Radiofrequency ablation ($n = 236$)	Hepatic resection ($n = 138$)	P
Demography			
Men:women	145:91 (38.6%)	101:37 (26.8%)	0.0021
Age (median, range)	67 (38–87)	62.5 (29–80)	< 0.001
Decompensated cirrhosis	16 (6.8%)	5 (3.6%)	0.20
HBsAg	24 (10.2%)	46 (33.3%)	< 0.001
Antibody to HCV	197 (83.5%)	84 (60.9%)	< 0.001
History of alcohol intake > 500 kg	21 (8.9%)	16 (11.6%)	0.40
Observation period (year)	3.7 (0.1–9.9)	4.5 (0.1–10.0)	0.041
Laboratory data (median, range)			
ICG R15 (%)*	28 (1–100)	21 (3–68)	< 0.001
Bilirubin (mg/dl)	1.0 (0.2–3.1)	1.0 (0.3–2.2)	0.003
Albumin (g/dl)	3.5 (2.2–4.2)	3.6 (2.8–4.4)	< 0.001
Aspartic transaminase (IU)	55 (17–311)	45 (17–386)	0.006
Platelet count ($\times 10^3/mm^3$)	97 (19–253)	127 (38–272)	< 0.001
Prothrombin time (%)	84 (31–125)	91 (59–115)	0.001
Liver cancer			
Median size (mm)	18 (8–30)	20 (5–30)	< 0.001
Single/multiple	195/41 (17.4%)	114/24 (17.4%) 1.00	
α-fetoprotein (ng/ml)	19 (1–2080)	17 (1–2610)	0.84
PIVKA-II (AU/L)†	17 (7–1470)	20 (9–1650)	0.008

*ICG R15, indocyanine green retention rate at 15 min.

†PIVKA-II, protein induced by vitamin K antagonist-II.

HCV, hepatitis C virus.

Treatment for initial hepatocellular carcinoma

Physicians and surgeons usually held a conference about the choice of therapy in individual patients. RFA or surgical therapy were selected considering the site, size and number of tumours, liver function and the patient's general status. Both RFA and the surgical procedure were explained fully to all the patients, and informed consent was obtained. Despite the feasibility and availability of surgery, some patients voluntarily preferred RFA under informed consent.

Radiofrequency ablation therapy was performed percutaneously under US or CT guidance, under conscious sedation with fentanyl citrate (0.1–0.2 mg, Fentanyl; Daiichi-Sankyo, Tokyo, Japan) or pethidine hydrochloride (35–70 mg, Opystan; Tanabe-Mitsubishi, Osaka, Japan) administered intravenously. RFA was performed using three kinds of apparatus: a radiofrequency interstitial tumour ablation system (RITA, RITA Medical Systems Inc., Mountain View, CA, USA), a cool-tip system (Tyco Healthcare Group LP, Burlington, VT, USA) and a radiofrequency tumour coagulation system (RTC system, Boston-Scientific Japan Co., Tokyo, Japan).

Hepatic resection was performed under intra-operative US monitoring and guidance. In the cases of small and superficial HCC, arterial and portal vein clumping at the hepatic hilum was not usually performed for maintenance of liver perfusion.

Evaluation of the therapeutic effect

To evaluate the efficacy of local ablation, a dynamic CT was performed at 2–7 days after treatment with RFA, and 8–21 days after surgery. CT findings were confirmed by consensus among at least two hepatologists and radiologists. On dynamic CT images, the non-enhancing area was measured as the ablated area. When the diameter of the non-enhancing area was greater than that of the ablated nodule, RFA was considered to have had a complete effect, and the treatment was terminated. When patients had a smaller ablated area or a positively enhanced area in the original tumour based on CT results after RFA therapy, they usually underwent an additional RFA within several days.

Follow-up of patients

Physicians observed the patients every 4–8 weeks after the first treatment. Liver function test, haematology and tumour markers were measured every 1–2 months. After the completion of eradication of HCC, recurrence was surveyed with CT or magnetic resonance imagings (MRI) every 3–4 months. Serum α-fetoprotein (AFP) and des-γ-carboxy prothrombin were also measured every 1–2 months to detect recurrence as early as possible.

During a median observation period of 4.2 years, four patients (1.1%) were lost to follow-up.

Statistical analysis and the Markov model

Standard statistical measures and procedures were used. The χ^2-test, Fisher's exact test and Mann–Whitney's *U*-test were used to analyse the differences in the demography, laboratory findings and tumour characteristics between the RFA group and the surgery group. The recurrence rate, progression rates and survival rate were analysed using the Kaplan–Meier technique (16) with the log-rank test. Cox's proportional hazard analysis was performed to evaluate independent predictors of the outcomes.

The Markov model (17) was adopted to analyse the transition rates from the early stage to the intermediate stage of HCC, intermediate to advanced stage and advanced stage to death. A homologous Markov chain consisted of four states (Fig. 1). These were the early stage of HCC (solitary or multiple up to three nodules, 3 cm or less each), the intermediate stage (four nodules or more, or larger than 3 cm), the advanced stage (portal vein

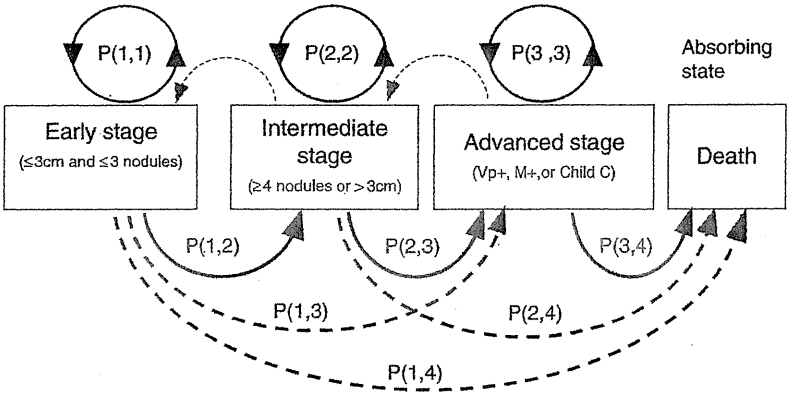

Fig. 1. The Markov state transition diagram of hepatocellular carcinoma. Four states were defined: early stage (solitary or multiple up to three nodules, 3 cm or less in diameter each), intermediate stage (multiple nodules of four or more, or 3.1 cm or more), advanced stage (main portal vein invasion, extrahepatic metastasis or Child–Pugh C) and death. Of these, death was the absorbing state from which no transitions to the other states occurred. The transition in one cycle (1 year) is shown. Arrows connecting two different states indicate the transitions observed.

invasion, extrahepatic metastasis or Child–Pugh score C) and death as an absorbing state from where no transitions to the other states occurred. The model was based on the following principles: (i) the four states are mutually exclusive and collectively exhaustive; (ii) the Markov assumption for the current state without any memories of prior states; (iii) time intervals are uniform; and (iv) transition probabilities are constant and time independent. Items (i) and (ii) define a Markov chain, whereas items (iii) and (iv) characterize a homogenous Markov chain (18).

A *P*-value of < 0.05 in a two-tailed test was considered significant. Data analysis was performed using the computer program IBM SPSS STATISTICS ver. 18 (19).

Results

Effect of initial treatment

After the initial session of RFA or surgery, complete ablation for entire tumour nodules was obtained in 232 patients (98.3%) in the RFA group and in 138 patients (100%) in the surgery group. Among four patients (1.7%) with incomplete ablation after the initial session of RFA, two achieved complete necrosis by re-RFA performed after a few months, and the other two underwent TACE for the residual tumour nodules.

Complications of treatment (Table 2)

After the initial therapy with RFA or surgery, 12 patients developed major complications after treatment: seven in the RFA group and five in the surgery group. There was no treatment-related death within 6 months after therapy in any of the patients in the RFA and surgery groups. Although abdominal pain, mild aggravation of liver function test, low-grade fever, transient elevation of aminotransferases and bilirubin values were often found after RFA therapy, significant deterioration of performance status and prolonged stay in the hospital were not observed.

Cumulative recurrence rates and treatment for recurrent hepatocellular carcinoma

The initial recurrence rates were compared between the two groups according to the initial therapy. The initial recurrence rates after treatment in the RFA and the surgery group were 11.3 and 14.2% at the end of the first year, 40.4 and 29.3% in the second year, 53.3 and 40.6% in the third year, 65.0 and 48.8% in the fourth year and 69.5 and 53.7% in the fifth year respectively. The recurrence rate in the RFA group was significantly higher than that of the surgery group (log-rank test, $P = 0.015$) (Fig. 2).

For the treatment of a recurrent tumour, we fundamentally adopted RFA or surgical treatment when patients had an early stage of HCC with sufficient liver function. Although initial therapy included surgery, patients with a recurrent tumour tended to receive RFA therapy more frequently. When a tumour progressed to the intermediate stage with a large tumour and/or multiple nodules, TACE was usually performed using anti-tumour agents, iodinated poppy seed oil fatty acid (Lipiodol Ultra-Fluide™, Guerbet Japan, Tokyo) and gelatin sponge particles. When the tumour progressed to the advanced stage (portal invasion, extrahepatic metastasis, or Child–Pugh C) during repeated local ablation or TACE therapy, anti-tumour therapy was usually not performed, except for systemic or intra-arterial chemotherapy. Anti-molecular targeted agents were not available during the study period in Japan.

Table 2. Complications after the initial treatment

Complication	Initial therapy: Radiofrequency ablation ($n = 236$)	Initial therapy: Hepatic resection ($n = 138$)
Perforation of jejunum	2	0
Biloma and/or biliary infection	3	1
Prolonged ascites	1	2
Jaundice	0	1
Haemorrhage requiring transfusion	1	1

Cumulative progression rates from the early to the intermediate stage

A total of 98 (26.2%) developed to the intermediate stage during the observation: 65 (27.5%) in the RFA group and 33 (23.9%) in the surgery group.

Crude development rates to the intermediate stage in the RFA and surgery groups were 18.2 and 13.0% in the third year, 33.1 and 22.1% in the fifth year, and 40.9 and 31.8% in the fifth year respectively. The development rate of the RFA group was slightly higher ($P = 0.14$) (Fig. 3a).

Independent factors associated with the stage development rate were explored in the patients. Multivariate hazard analysis showed that the rate is independently associated with positive HBsAg ($P = 0.041$) and a high platelet count ($P = 0.032$). The factor of initial therapy

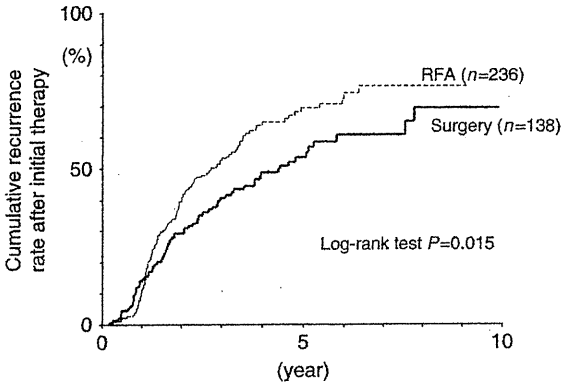

Fig. 2. Cumulative recurrence rates after therapy in patients with an early stage of hepatocellular carcinoma, according to initial therapy. RFA, radiofrequency ablation.

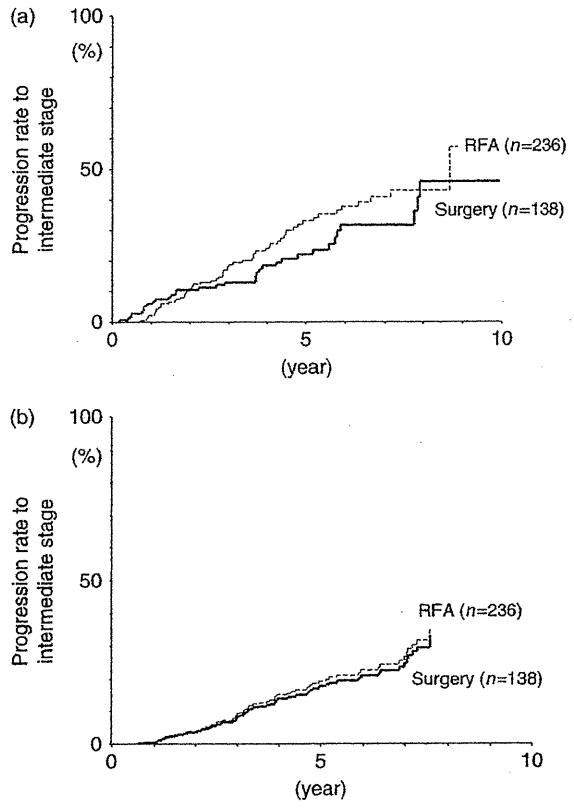

Fig. 3. (a) Crude development rates to the intermediate stage of hepatocellular carcinoma according to initial therapy. (b) Adjusted development rates to the intermediate stage, using proportional hazard analysis. RFA, radiofrequency ablation.

did not affect the eventual survival rate (hazard ratio 1.09, $P=0.70$) (Table 3).

Cumulative progression curves from the early stage to the intermediate stage were drawn from the multivariate analysis in an imaginary RFA group and an imaginary surgery group, with an average positive rate of HBsAg and an average platelet count (Fig. 3b). Five-year progression rates to the intermediate stage were 19% in the RFA group and 18% in the surgery group. The differences in the progression rates were considered as a 'pure' impact of the difference in the initial mode of therapy on future stage progression, which was adjusted with significant covariates assuming a standardized study group.

Survival rates and predictive factors

A total of 87 (23.3%) died during the observation: 60 (25.4%) in the RFA group and 27 (19.6%) in the surgery group.

The crude survival rates in the RFA group and the surgery group were 88.5 and 92.6% in the third year, 71.7 and 80.9% in the fifth year and 60.6 and 74.6% in the seventh year respectively (Fig. 4a). The survival rate of the surgical therapy group was higher but statistical significance was not obtained ($P=0.071$).

Table 3. Independent factors associated with the progression rate from an early stage to an intermediate stage of hepatocellular carcinoma

Factors	Category	Hazard ratio (95% confidence interval)	*P*
HBsAg	1: negative	1	
	2: positive	0.41 (0.20–0.82)	0.012
Platelet count	1: ≥ 100 000/mm^3	1	
	2: < 100 000/mm^3	1.58 (1.04–2.39)	0.032
Initial therapy	1: surgery	1	
	2: RFA	1.09 (0.69–1.71)	0.70

RFA, radiofrequency ablation.

Independent factors associated with survival were explored in all the patients. Multivariate hazard analysis indicated that the survival rate is independently associated with a positive HBsAg ($P=0.038$), a low indocyanine green retention rate at 15 min (ICG R15) ($P<0.001$) and a low AFP value ($P=0.021$). The factor of initial therapy did not affect the eventual survival rate (hazard ratio 1.26, $P=0.35$) (Table 4).

Overall survival curves in patients with an early stage of HCC were drawn from the multivariate analysis in an imaginary RFA group and an imaginary surgery group, using an average positive rate of HBsAg, an average ICG R15 value and an average AFP value (Fig. 4b). Five-year survival rates were estimated as 80% in the RFA group and 81% in the surgery group, and 7-year rates were 71 and 72% respectively. Among 87 deaths during the observation, 70 (80.5%) died from progression of HCC, 14 (16.1%) died from liver failure without progression of HCC and the remaining three patients died from causes other than liver disease

Probabilities for transition among four disease states of hepatocellular carcinoma

The Markov model for the progression of HCC depended on the probabilities for transition among the four states at one time interval that was set at 1 year. Yearly transition probabilities were calculated based on 1892 person-year data from the 374 patients with an early stage of HCC. Figure 5 illustrates a probability diagram of the long-term progression of HCC calculated from the Markov model. All patients were at an early stage initially, but intermediate and advanced stages gradually increased with time. Approximately half of the patients died, and < 40% of the patients remained at early stage at the end of the 10th year.

The results are shown in Table 5 as a matrix of the transition probabilities for three subsets composed of three decades of their lives (< 60, 60–69 and ≥ 70 years) stratified by four states (early stage, intermediate stage, advanced stage and death).

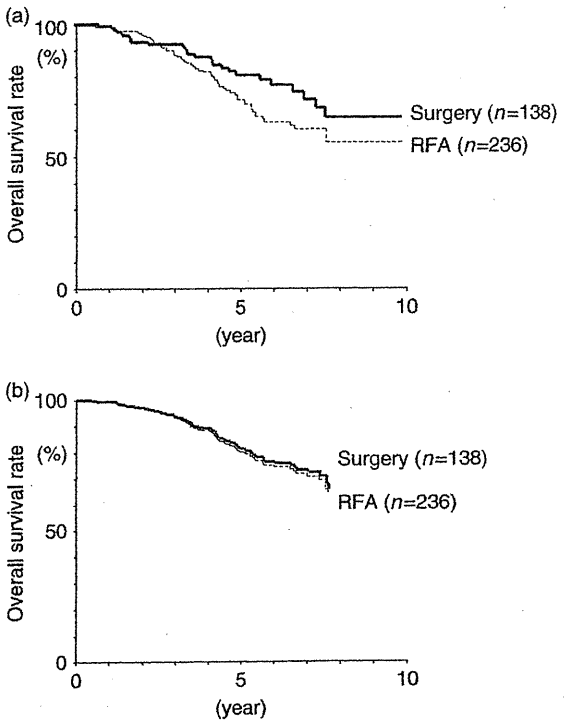

Fig. 4. (a) Crude survival rates in patients receiving radiofrequency ablation and those undergoing surgery as the initial therapy. (b) Adjusted survival rates in the radiofrequency group and surgery group, using proportional hazard analysis. RFA, radiofrequency ablation.

Table 4. Independent factors associated with the survival rate after the initial treatment for hepatocellular carcinoma

Factors	Category	Hazard ratio (95% confidence interval)	*P*
HBsAg	1: negative	1	
	2: positive	0.43 (0.19–0.94)	0.034
ICG R15*	1: < 30%	1	
	2: ≥30%	1.96 (1.20–3.20)	0.0070
α-fetoprotein	1: < 40 mg/ml	1	
	2: ≥40 mg/ml	1.71 (1.09–2.68)	0.020
Prothrombin time	1: < 80%	1	
	2: ≥80%	0.60 (0.37–0.96)	0.035
Initial therapy	1: surgery	1	
	2: RFA	1.09 (0.66–1.81)	0.73

*ICG R15, indocyanine green retention rate at 15 min.
RFA, radiofrequency ablation.

In the matrix of age of < 60 years, 2.34% of the patients in the early stage developed to the intermediate stage annually, 1.40% to the advanced stage and 0.93% died. The remaining 95.33% of the patients remained in the early stage after 1 year. The probability for the transition from an early stage to an intermediate stage

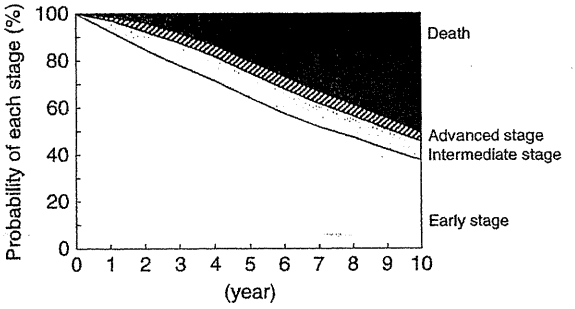

Fig. 5. Illustrated transition probabilities of patients, from the early stage of hepatocellular carcinoma, to the intermediate stage, the advanced stage and to death.

Table 5. One-year state-transition probability matrices for subsets of hepatocellular carcinoma*

	Early	Intermediate	Advanced	Death
All Patients of all age groups				
Early	92.17	4.81	1.73	1.29
Intermediate		69.32	27.27	3.41
Advanced			24.77	75.23
Death				100.00
Age < 60 years				
Early	95.33	2.34	1.40	0.93
Intermediate		58.33	37.50	4.17
Advanced			23.53	76.47
Death				100.00
Age 60–69 years				
Early	91.40	5.90	1.35	1.35
Intermediate		68.18	30.30	1.52
Advanced			22.21	78.79
Death				100.00
Age ≥70 years				
Early	90.68	5.49	2.33	1.50
Intermediate		74.42	22.09	3.49
Advanced			27.91	72.09
Death				100.00

*Early stage, solitary or multiple up to three nodules 3 cm or less each; Intermediate stage, four nodules or more, or larger than 3 cm; Advanced stage, portal vein invasion, extrahepatic metastasis, or Child–Pugh score C.

was significantly lower in young patients < 60 years of age (2.34%) than that in patients 60 years of age or older (5.70%)($\chi^2 = 7.76$, $P = 0.0053$). From the matrix stratified by three age groups, the transition probability from an intermediate to an advanced stage decreased with age: 37.50% in patients < 60 year of age, 30.30% in patients 60–69 year of age and 22.09% in patients 70 year of age or older ($\chi^2 = 10.57$, $P = 0.0011$).

Probabilities for transition according to the initial treatment

We also evaluated the transition probabilities among the four states in the subgroups of RFA and surgery as the initial mode of therapy.

In the matrix of patients receiving RFA therapy, the transition probability from early to intermediate stage was 5.40%, probability to the advanced stage was 1.63% and to death was 1.73%. In the patients undergoing surgery, the transition probability from an early to an intermediate stage was 3.90%, probability to an advanced stage was 1.87% and to death was 0.62%.

The probability for the transition from an early stage to an intermediate stage was slightly higher in the RFA group (5.40%) than that in the surgery group (3.90%), but statistical significance was not found ($\chi^2 = 1.90$, $P = 0.17$).

Discussion

Radiofrequency ablation has been considered as a less curative mode of therapy than surgical resection, because local tumour progression sometimes occurs after conservative treatment with relatively small ablative margins. As those patients with loco-regional therapy are generally followed up for tumour recurrence with a short time interval of 3–6 months using CT or MRI, we can usually ablate a newly appeared or a locally progressed tumour within a small size and few numbers. In order to elucidate the efficacies and usefulness of RFA compared with surgical resection, we analysed many HCC patients receiving RFA or surgical therapy regarding tumour progression and survival.

Fortunately, in Japan, where highly socialized medicine is practiced with everyone covered by some form of health insurance, almost all of the patients can receive any extensive medical services including surgery, RFA, embolization and repeated imaging diagnosis, regardless of the cost. Under intensive check-up and treatment repetition, the Markov model showed the probability of remaining at the early stage as 92.17% per year: the transition rate from the early to the intermediate stage was 4.81%, to the advanced stage 1.73 and to death 1.29% respectively. Similarly, the probabilities of remaining at the intermediate and advanced stages were 69.32 and 24.77% per year respectively.

Because young patients with HCC usually have better liver function and a relatively low carcinogenesis rate, younger patients are more likely to undergo radical methods of therapy for a recurrent tumour repeatedly. The reason for the low transition rate from the early to the intermediate stage was convincingly explained in the young patient group (Table 4). In contrast, the transition rate from the intermediate to the advanced stage was significantly higher in the young patient group. Although the exact reason was not known, multiple tumours of younger patients possibly progressed rapidly or were resistant to TACE. Hence, the Markov model would be eligible for simulating the outcomes of patients with the early stage of HCC. It is also helpful in planning strategies for the management of small HCC, for the eventual prolongation of a patient's life and for ideal cost-effective guidelines on a national basis, not only in Japan but also elsewhere in the world where the prevalence of HCC is increasing. Although we once generated a 'five-state model' consisting of no tumour, early stage, intermediate stage, advanced stage and death, we finally adopted the current 'four-state model' because of good mathematical fit and statistical robustness. Molinari and Helton (20) and Cho *et al.* (21) described a progression model of HCC after RFA and/or hepatectomy by the Markov model. Both authors performed a meta-analysis-like study using heterogeneous sources of patients from varied published articles, and estimated progression models of HCC in hypothetical patient cohorts. We analysed the actual clinical courses of patients in a single institution, where the same diagnostic and therapeutic procedures were adopted for every patient. Sufficient medical procedures and resources under a universal medical insurance system of the country seemed to give rise to better outcomes and survival, but an exact comparison cannot be carried out using the current data and the previous literatures.

In this study, we also compared RFA and surgery as an initial therapy for the early stage of HCC. Understandably, older patients, patients with severe cirrhosis and those with a concomitant disease other than liver disease tended to undergo non-surgical therapy. In addition, young patients with HBV-related HCC were likely to receive surgery because of good liver function, relatively low potential of recurrence and young age. Although the crude recurrence rate and the crude progression rate from the early stage to the intermediate stage were higher in patients receiving RFA therapy, multivariate analysis with adjustment of background biases showed that the initial mode of therapy did not affect the progression rate and did not affect the overall survival rate. When a regular check-up of imagings with an interval of 3–4 months was conducted, an additional ablation therapy was usually performed successfully for a small locally progressed tumour. Under intensive medical care for liver disease, the initial mode of therapy therefore did not affect the overall survival of a patient with an early stage of HCC. When a careful check-up with imagings and adequate application of repeated ablative procedures for HCC were performed, the choice of ablative manners was insignificant compared with the background liver features of aetiology of liver disease (hepatitis virus) and severity of liver disease (platelet count). The choice of ablative therapy for small-sized HCC should also be assessed from the viewpoints of conservation of liver function, cost-effectiveness and quality of life (9, 10, 12, 22).

Since it seemed to require at least 5 years to obtain a statistical difference in the recurrence rates and survival rates between RFA-treated and surgically treated groups, a prospective randomized trial is actually difficult to perform from both ethical and medical viewpoints. One of the significant results of the current study is that highly socialized medical circumstances with sufficient medical practice can attain a high survival rate of 71–80% at the end of the fifth year in patients at an early stage.

Further studies are required to determine the relationship between patient's age and stage transition. Because HCV-related chronic hepatitis often progresses to HCC during the clinical course, this kind of staging model with analyses of medical intervention will be necessary in the future from the viewpoints of epidemiological assessment and medical politics, together with patient's quality of life and feeling of satisfaction.

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Hepatology Research

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Original Article

Efficacy and safety in sitagliptin therapy for diabetes complicated by chronic liver disease caused by hepatitis C virus

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Aim: Diabetes is present in patients with chronic liver disease caused by hepatitis C virus (HCV). The aim of this case–control study is to assess the efficacy and safety of dipeptidyl peptidase-4 inhibitor (sitagliptin) for type 2 diabetes mellitus (T2DM) with chronic liver disease caused by HCV.

Methods: Sixteen HCV positive patients with T2DM treated by sitagliptin were retrospectively enrolled. These patients were given sitagliptin between December 2009 and January 2010. Another 16 HCV patients with T2DM treated only with diet and excise for 48 weeks were selected as the control group. Serum levels of fasting plasma glucose (FPG), hemoglobin A1C (HbA1C), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured before and 12, 24, 36 and 48 weeks after the initiation of treatment.

Results: In the sitagliptin group, the average HbA1C level decreased approximately 0.8% at 48 weeks after the initiation of sitagliptin. Next, the average FPG level decreased approximately 20 mg/dL during follow up after the initiation of sitagliptin. All the patients were able to take sitagliptin of 50 mg/day without reduction because of sitagliptin-related side-effects. On the other hand, in the control group, the average HbA1C and FPG level did not change with statistical significance during follow up of 48 weeks. Regarding aminotransferase, there were no significant changes of average AST and ALT level during follow up of 48 weeks in both the sitagliptin group and control group.

Conclusion: Our results indicate that sitagliptin is effective and safe for the treatment of T2DM complicated with HCV positive chronic liver disease.

Key words: hepatitis C virus, sitagliptin, type 2 diabetes mellitus

INTRODUCTION

HEPATITIS C VIRUS (HCV) is one of the more common causes of chronic liver disease in the world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20–50% of cases over a period of 10–30 years.[1,2] In addition, HCV is a major risk for hepatocellular carcinoma (HCC).[3–7] Lately, it has been reported that chronic HCV infection is associated with type 2 diabetes mellitus (T2DM)[8–14] Moreover, T2DM has been suggested to enhance with the development of HCC and poor prognosis of liver transplantation.[15–19] Thus, in patients with chronic liver diseases, the management of T2DM is very important to improve the prolonged prognosis.

However, most oral hypoglycemic agents are metabolized in the liver and often induce the liver damage. Thus, it is difficult to treat the patients who have T2DM complicated with chronic liver disease.[20] A new oral hypoglycemic agent, dipeptidyl peptidase-4 (DPP-4)

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inhibitor (sitagliptin), is minimally metabolized.[21,22] Hence, sitagliptin raises the possibility for use in patients with T2DM complicated with chronic liver disease.

With this background in mind, the case–control study was initiated to investigate the efficacy and safety of DPP-4 inhibitors for T2DM patients with HCV positive chronic liver disease.

METHODS

Patients

SIXTEEN PATIENTS WITH T2DM complicated with HCV positive chronic liver disease started the treatment with oral DPP-4 inhibitor (sitagliptin; MDS, Tokyo, Japan) of 50 mg/day from December 2009 to January 2010 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. These 16 consecutive patients treated with sitagliptin of 50 mg/day were regarded as the sitagliptin group. Inclusion criteria of DPP-4 inhibitor administration were as follows: (i) evidence of diabetes mellitus (i.e. plasma glucose concentration of ≥126 mg/dL [6.9 mM) in the fasting state, ≥200 mg/dL [11.0 mM] in casual state and/or 2 h after a 75-g oral glucose load; (ii) a diabetic history of less than 2 years; (iii) features of chronic hepatitis or cirrhosis diagnosed by ultrasonography and/or computed tomography; (iv) positive for serum HCV RNA; (v) negativity for hepatitis B surface antigens (HBsAg), antinuclear antibodies or anti-mitochondrial antibodies in serum, as determined by radioimmunoassay or spot hybridization; (vi) no evidence of HCC nodules as shown by ultrasonography and/or computed tomography; and (vii) no underlying systemic disease, such as systemic lupus erythematosus and rheumatic arthritis. The distinction between chronic hepatitis and liver cirrhosis in patients was done by discriminant function using platelet, hyaluronic acid, and γ-globulin.[23] Patients with either of the following criteria were excluded from the study: (i) they were taking medicines except DPP-4 inhibitors known to alter glucose tolerance; and/or (ii) they had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial. Patients in the sitagliptin group exercised and participated in diet therapy in addition to administration of sitagliptin. In the same period, 303 patients with T2DM and chronic liver disease type C were not treated with antidiabetic drugs. These patients exercised and participated in diet therapy for T2DM. Seventy-three of these 303 patients were applied with seven inclusion criteria and two exclusion criteria as described above. Sixteen subjects in the control group were selected from these 73 patients by matching 1:1 with the sitagliptin group for age and sex. Patients who belonged to the control group or sitagliptin group had been subjected to lifestyle intervention of diet and physical exercise after the diagnosis of T2DM. The diet prescription included daily calorie intake of 125.6 kJ/ideal body-weight (kg), a protein energy fraction of 15% and a fat energy fraction of 25%. Physical activity was recommended as at least 120 min of aerobic exercise a week. The physicians in charge explained the methods and side-effects of sitagliptin therapy to each patient and/or patient's family before sitagliptin therapy. Informed consent was obtained from 16 patients of the sitagliptin group before the initiation of sitagliptin therapy. All of the studies in the control group were performed retrospectively by collecting and analyzing data from the patient records. This study was approved by the Institutional Review Board of Toranomon Hospital.

Outcome measures

Type 2 diabetes mellitus was diagnosed by the 2003 criteria of the American Diabetes Association:[24] (i) casual plasma glucose of 200 mg/dL or more; (ii) fasting plasma glucose (FPG) of 126 mg/dL or more; and/or (iii) 2-h post-glucose (oral glucose tolerance test) of 200 mg/dL or more. Hemoglobin A1c (HbA1c) was measured using a high-performance liquid chromatography method.

Laboratory investigation

Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL, USA). HCV RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test v2.0; Roche, Tokyo, Japan). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI, USA). The value for HbA1c (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA1c (%) = HbA1c (Japan Diabetes Society, JDS) + 0.4%, considering the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard materials and measurement methods and HbA1c (NGSP).[25] Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (kg) / height (m^2).

Follow up

The starting time of follow up in the sitagliptin group was the initiation of sitagliptin therapy. That is, the time

Table 1 Clinical characteristics at the starting time of follow up

	Sitagliptin group	Control group	*P*-value
n	16	16	
Age (years)	65.3 ± 9.1	65.2 ± 9.5	1.0
Sex (male/female)	8/8	8/8	1.0
Chronic hepatitis/liver cirrhosis	13/3	13/3	1.0
BMI	23.0 ± 3.5	23.5 ± 2.9	0.713
BMI (post-intervention)	22.4 ± 2.4	22.6 ± 2.3	1.0
AST (IU/L)	43 ± 34	34 ± 21	0.170
ALT (IU/L)	45 ± 31	40 ± 31	0.423
Albumin (g/dL)	3.8 ± 0.4	3.9 ± 0.4	0.873
Total bilirubin (mg/dL)	0.9 ± 0.5	0.8 ± 0.3	0.167
Platelets ($\times 10^4/mm^3$)	15.1 ± 5.3	17.0 ± 6.7	0.208
Hyaluronic acid (ng/mL)	132 ± 80	112 ± 62	0.637
HbA1c (NGSP value)	7.4 ± 0.8	7.2 ± 0.9	0.552
FPG (mg/dL)	142.1 ± 24.1	140.0 ± 25.7	0.951

Data are number of patients or mean ± standard deviation.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; HCV, hepatitis C virus; NGSP, National Glycohemoglobin Standardization Program.

was from December 2009 to January 2010. The starting time of follow up in the control group was the same as that in the sitagliptin group. Patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check up. An overnight (12 h) fasting blood sample and HbA1C sample were taken for routine analyses. These included transaminase activities.

Statistical analysis

Clinical differences between the sitagliptin group and control group were evaluated by Wilcoxon rank sum test or Fisher's exact test. Changes in serum HbA1C and FPG level between the sitagliptin group and control group during follow up were analyzed by one-way repeated measurement ANOVA. Next, predictive factors for responders were assessed. A $P < 0.05$ was considered to be statistically significant. SPSS ver. 11.5 for Windows was used to perform statistical analysis.

RESULTS

Patients' characteristics

TABLE 1 SHOWS the characteristics before follow up in the 32 patients with T2DM and HCV positive chronic liver disease. There were no significant differences in clinical profiles between the sitagliptin group and control group.

Change of HbA1c and FPG

Change of average HbA1C and FPG level are plotted in Figures 1 and 2 in the sitagliptin group and control group. In the sitagliptin group, average HbA1C level decreased from 7.4% to 6.5% at 48 weeks after the initiation of sitagliptin. Moreover, average FPG level could be deduced at approximately 20 mg/dL during follow up after the initiation of sitagliptin. The HbA1C and FPG level in the sitagliptin group were statistically lower than those in the control group.

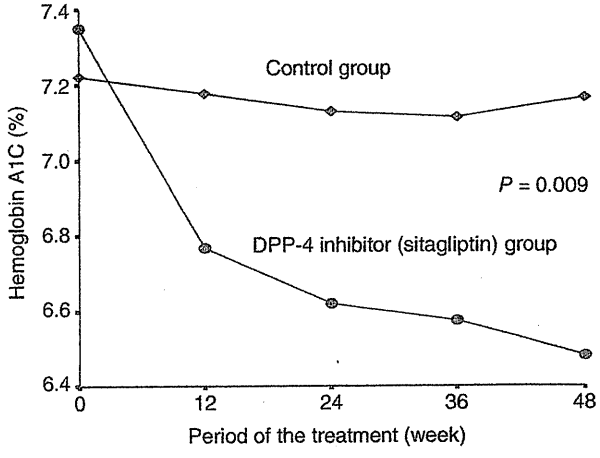

Figure 1 Change of average hemoglobin A1c (HbA1c) level during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group.

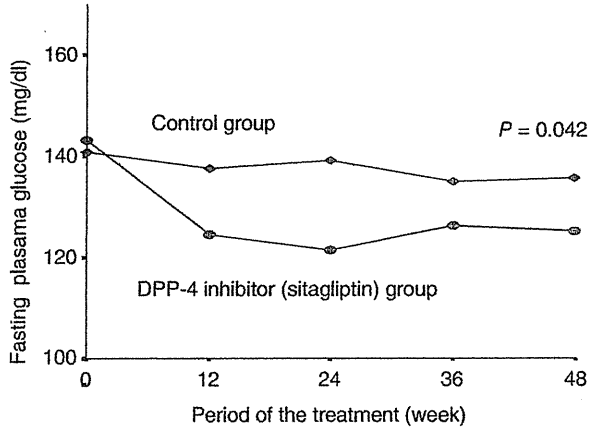

Figure 2 Change of average fasting plasma glucose during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group.

Adverse events of sitagliptin

Regarding side-effects, none of the patients treated with DDP-4 inhibitor had sitagliptin-related episodes severe enough to stop the treatment of sitagliptin. Thus, all the patients were able to take sitagliptin 50 mg/day for 48 weeks without reduction. Next, changes of average AST and ALT level during follow up are plotted in Figure 3. There were no significant changes of average AST and ALT level during follow up in either the sitagliptin or control group.

DISCUSSION

WE HAVE DESCRIBED the efficacy and side-effects of sitagliptin for T2DM patients with HCV positive chronic liver disease in the present study. The present study was limited by being a case–control study. Another limitation of the study was that patients were treated with different types of diet and different excise. This heterogeneity makes it slightly difficult to interpret the results of the study.

On the other hand, the present study shows several findings with regard to the efficacy and side-effects of sitagliptin for T2DM patients with HCV positive chronic liver disease. First, in the sitagliptin group, average HbA1C and FPG levels after the initiation of sitagliptin were statistically lower than those at the starting time of DPP-4 inhibitor. It is suggested that sitagliptin increases active glucagon-like peptide-1, stimulates insulin secretion and inhibits glucagon secretion.[21,22] Thus, it is accepted that sitagliptin could improve both HbA1C and glucose level in patients with T2DM and HCV positive chronic liver disease.

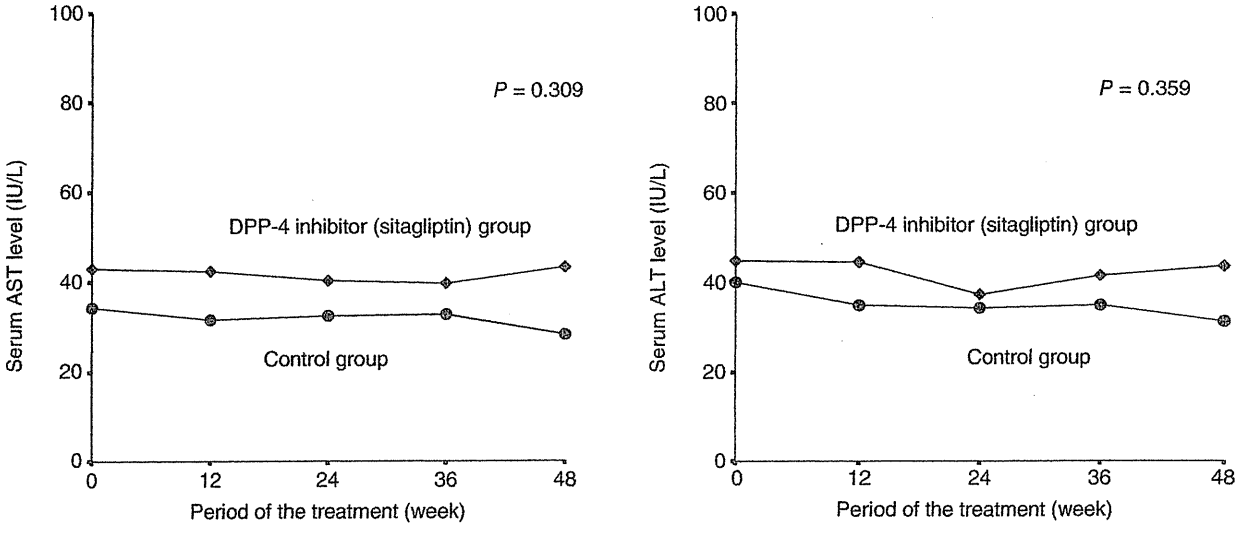

Figure 3 Change of average aminotransferase level during follow up was plotted in both the dipeptidyl peptidase-4 (DPP-4) inhibitor group and control group. (a) Change of average aspartate aminotransferase (AST) level during follow up was plotted in both the DPP-4 inhibitor group and control group. (b) Change of average alanine aminotransferase (ALT) level during follow up was plotted in both the DPP-4 inhibitor group and control group. Patients who belonged to the control group or sitagliptin group were subjected to lifestyle intervention of diet and physical exercise. The diet prescription included daily calorie intake of 30 kcal/ideal bodyweight, a protein energy fraction of 15% and a fat energy fraction of 25%.

Second, administration of sitagliptin is minimal risk and highly tolerable for T2DM patients with HCV positive chronic liver disease. In the present study, none of the patients treated with DDP-4 inhibitor had sitagliptin-related episodes severe enough to stop the sitagliptin therapy. Thus, all the patients could take sitagliptin of 50 mg/day over 48 weeks without reduction or stopping. This new oral hypoglycemic agent, sitagliptin, is minimally metabolized and over 80% of it is excreted in the urine. It seems not to alter pharmacokinetics in hepatic insufficiency.[22] Thus, sitagliptin has few possibilities to cause the aggravation of the chronic liver damage. In fact, in the present study, three patients with liver cirrhosis did not have elevation of aminotransferase during the treatment by sitagliptin. This result indicates that sitagliptin is valuable for treating T2DM with HCV positive liver cirrhosis.

Type 2 diabetes mellitus has been increasing dramatically in many nations including Japan over the past decades.[26] It is widely accepted that approximately 7–8 million people are affected by DM in Japan. Approximately 8–10% of adults in Japan have T2DM. Recently, it has been reported that T2DM has occurred in HCV positive chronic liver disease.[8–13] Moreover, HCV patients with T2DM are at major risk for HCC.[15–17] So, in patients with T2DM and HCV positive chronic liver diseases, the management of DM is very important to improve the prolonged prognosis. However, most oral hypoglycemic agents (thiazolidines, sulfonylurea and biguanides) are metabolized in the liver. Thus, it is suggested that most oral hypoglycemic agents often induce liver damage. The new oral hypoglycemic agent, DPP-4 inhibitor (sitagliptin), is minimally metabolized. Hence, this drug raises the possibility of being used for T2DM patients with HCV positive chronic liver disease.

In conclusion, our retrospective study suggests that sitagliptin is effective and safe for the treatment of T2DM complicated with HCV positive chronic liver disease.

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Common Genetic Polymorphism of ITPA Gene Affects Ribavirin-Induced Anemia and Effect of Peg-Interferon Plus Ribavirin Therapy

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An association between a single nucleotide polymorphism (SNP) in the inosine triphosphate pyrophosphatase (ITPA) gene and reduction of hemoglobin during peg-interferon plus ribavirin combination therapy for patients with chronic hepatitis C virus (HCV) infection has been reported. However, the effect of the SNP on outcome of therapy has not been fully elucidated. Factors associated with anemia during combination therapy, including rs1127354 genotype, were analyzed in 1,002 treated patients. The effect of the SNP on outcome of therapy was analyzed in a subset of 830 patients with genotype 1. A rapid initial decrease in hemoglobin levels was observed in patients with rs1127354 genotype CC compared with a slow decrease in non-CC patients. Cumulative reduction of ribavirin was significantly more frequent in genotype CC patients than non-CC patients (odds ratio 1.928, $P = 8.6 \times 10^{-8}$). The frequency of patients who received at least the recommended 80% of scheduled ribavirin was significantly lower among genotype CC patients, especially among those who had pretreatment hemoglobin levels between 13.5 and 15 g/dl ($P < 0.03$), and the sustained viral response rate was significantly lower in this group of patients. Independent predictive factors for sustained virological response included a SNP in the IL28B locus (rs809991), age, fibrosis, ITPA SNP rs1127354 as well as pretreatment hemoglobin levels. Our data suggests that measures to prevent anemia should be considered for patients who have pretreatment hemoglobin levels less than 13.5 g/dl or who have rs1127354 genotype CC and pretreatment hemoglobin levels between 13.5 and 15 g/dl. ***J. Med. Virol. 83:1048–1057, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: inosine triphosphate pyrophosphatase; single nucleotide polymorphism; peg-interferon; anemia; dose reduction

INTRODUCTION

Hepatitis C virus (HCV), a positive-strand RNA flavivirus, chronically infects 170 million people worldwide and is responsible for up to 300,000 deaths due

Abbreviations: HCV, hepatitis C virus; ITPA, inosine triphosphate pyrophosphatase; SNP, single nucleotide polymorphism.

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to progression to liver cirrhosis and hepatocellular carcinoma [Alter, 1995; Chevaliez and Pawlotsky, 2007]. Currently, peg-interferon plus ribavirin combination therapy (PEG-RBV) is the most effective treatment, but it is only effective in 50% of patients with genotype 1b, and the therapy has severe side effects often requiring dose modification or discontinuation [Hadziyannis et al., 2004]. However, there are several factors that may help predict outcome of therapy, including HCV genotype [Zeuzem et al., 1996], virus titer [Zeuzem et al., 1996; Dienstag and McHutchison, 2006], age, fibrosis of the liver, obesity, race, hepatic steatosis [Dienstag and McHutchison, 2006], LDL cholesterol, gamma-GTP [Akuta et al., 2007], insulin resistance [Romero-Gómez et al., 2005], amino acid substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the interferon sensitivity determining region (ISDR) of the NS5A protein [Enomoto et al., 1995a; Akuta et al., 2005]. A series of recent studies have also identified common genetic variants in the IL28B locus on chromosome 19 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009] that are strongly associated with outcome of combination therapy.

Ribavirin-induced anemia is a serious side effect of therapy which results in dose reduction of ribavirin and possibly of interferon as well. The precise mechanism of induction of anemia remains to be determined. Ribavirin-induced hemolytic anemia accompanied by an increase in reticulocyte counts has been reported to be associated with membrane oxidative damage as well as impairment of erythrocyte Na–K pump activity and increase in dithiotreitol-sensitive fraction, malondialdehyde, and methemoglobin levels [De Franceschi et al., 2000]. Treating patients with erythropoietin, which induces erythropoiesis and helps alleviate anemia, has been reported to be effective in preventing ribavirin dose reduction and leads to better therapy outcome [Dieterich et al., 2003].

Recently, single nucleotide polymorphisms (SNPs) in the inosine triphosphate pyrophosphatase (ITPA) locus have been found to be associated with anemia in patients treated with combination therapy [Fellay et al., 2010; Ochi et al., 2010; Thompson et al., 2010]. In Caucasian patients there are two SNPs that are associated with ITPA enzyme activity [Fellay et al., 2010; Thompson et al., 2010], although one of these SNPs appears to be absent in Japanese patients [Ochi et al., 2010]. Although the effect of the ITPA polymorphism on ribavirin-induced anemia has been clearly demonstrated by these studies, the effect of the SNP on outcome of therapy has not been fully explored. Our previous report suggested an association of the polymorphism with sustained virological response (SVR) [Ochi et al., 2010], whereas other reports found no association [Fellay et al., 2010; Thompson et al., 2010].

In the current study, 1,002 patients who were treated with peg-interferon 2b plus ribavirin combination therapy were analyzed to elucidate the precise effect of the ITPA SNP on hemoglobin reduction. A subset of 830 of the patients with genotype 1 were further examined to assess the effect of the SNP on therapy outcome. The results show that reduction of ribavirin was frequent among patients with low pretreatment hemoglobin levels (<13 g/dl) as well as those with the ribavirin-sensitive ITPA genotype (rs1127354 CC) and intermediate pretreatment hemoglobin levels (13.5–15 g/dl). Our results suggest that anemia-preventing measures, such as administration of erythropoietin, should be considered for patients likely to develop anemia.

MATERIALS AND METHODS

Patients

Data from 1,002 patients who were treated with peg-interferon alpha 2b and ribavirin combination therapy for chronic hepatitis C infection between December 2004 and January 2010 were collected from Toranomon Hospital (Tokyo) and hospitals belonging to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html) in Hiroshima, Japan. Patient profiles are shown in Table I. All patients tested positive for HCV RNA for more than 6 months and were negative for hepatitis B and HIV and showed no evidence for other liver diseases including alcoholic hepatitis, hemochromatosis, Wilson's disease, and autoimmune hepatitis. Patients received weekly injections of peg-interferon-alpha-2b at 1.5 g/kg body weight for 48 weeks, and ribavirin was administered orally. The amount of ribavirin was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, and 1,000 mg for >80 kg). Ribavirin dose was reduced when hemoglobin levels fell to 10 g/dl, and both peg-interferon and ribavirin were discontinued when hemoglobin levels dropped to <8.5 g/dl. Patients who remained positive for HCV RNA during the first 12 weeks of treatment but became negative by week 32 received extended administration of both drugs until 72 weeks. The successful endpoint of treatment was considered SVR, defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. A subset of patients showed transient response (TR), in which HCV RNA dropped to undetectable levels but then later rebounded. The remaining patients in which HCV RNA never became undetectable were considered non-responders (NVR). Histopathological diagnosis was made by pathologists at each hospital according to the criteria of Desmet et al. [1994]. All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA Levels

HCV RNA levels were measured throughout the course of therapy via RT-PCR using the original

TABLE I. Characteristics of Patients by ITPA rs1127354 SNP Genotype

	All patients	Patients with HCV genotype 1		
	Total (n = 1,002)	Total (n = 830)	CC (n = 628)	CA/AA (n = 202)
Age (years)	58 (51–64)	58 (51–64)	58 (52–64)	58 (51–64)
Sex (M/F)	539/463	448/382	328/300	120/82
Height (cm)	161 (154–168)	161 (154–168)	161 (154–168)	161 (155–168)
Weight (kg)	58.5 (52–67)	58.2 (52–66.2)	58.05 (51.8–66.45)	59 (52–65)
rs8099917 (TT/GT/GG)	720/253/25	585/222/20	437/174/15	148/48/5
rs12979860 (CC/CT/TT)	543/198/52	541/197/52	403/151/44	138/46/8
rs1127354 (CC/CA/AA)	753/227/22	628/183/19	628/0/0	0/183/19
Core70 (W/M/ND)	240/143/619	239/143/448	175/114/339	64/29/109
Core91 (W/M/ND)	217/168/617	216/168/446	168/123/337	48/45/109
ISDR (0–1/≥2/ND)	287/80/635	287/80/463	216/61/351	71/19/112
Fibrosis (1/2/3/4/ND)	252/191/124/29/401	252/190/124/29/230	194/138/90/23/179	58/52/34/6/51
Activity (0/1/2/3/ND)	9/252/280/42/419	9/251/280/42/248	6/187/213/31/191	3/64/67/11/57
WBC (/mm^3)	4,700 (3,900–5,600)	4,700 (3,900–5,600)	4,700 (3,900–5,530)	4,900 (4,000–5,942)
Plt ($\times 10^4$/mm^3)	15.6 (12.2–19.7)	15.4 (12.2–19.35)	15.3 (12.1–19.33)	15.9 (12.45–19.4)
Hb (g/dl)	14 (13.2–14.9)	14 (13.2–14.9)	14.1 (13.2–14.9)	14 (13.4–15)
AST (IU/L)	45 (34–66)	45 (34–66)	45 (34–67)	45.5 (34–64.5)
ALT (IU/L)	53 (36–85)	53 (36–85)	52 (36–84.5)	55 (34.5–85)
γGTP (IU/L)	40 (25–73)	40 (25–73)	39.5 (25–72)	43.5 (25.25–77.25)
Total cholesterol (mg/dl)	172 (151–193)	172 (151–193)	172 (150–194)	171 (154–190)
HDL cholesterol (mg/dl)	51 (40–64)	51 (40–64)	52 (40.25–64)	50 (38–63.75)
Fasting blood sugar (mg/dl)	98 (89–112.8)	98 (89–113)	99 (89–113)	95 (88–108)
Virus titer (log IU/ml)	6.5 (6–7)	6.5 (6–7)	6.5 (6–7)	6.5 (6.1–6.9)
Viral genotype (1b/1a/others)	814/9/179	814/9/7	618/6/4	196/3/3
RBV treatment period (weeks)	48 (37–59)	48 (37–59)	48 (34.75–57)	48 (47–64.75)
RBV reduction (no/yes/ND)	316/450/236	315/448/67	212/366/50	103/82/17
Weeks to first RBV reduction	16 (5–48)	16.5 (5–48)	12 (4–47)	44 (12–51.75)
Outcome of therapy (NR/TR/SVR)	154/157/283	154/156/281	125/120/202	29/36/79

ND, not determined or data unavailable.
Categorical variables are reported as counts, and continuous variables are reported as median and interquartile range.

Amplicor method, the high range method, or the TaqMan RT-PCR test. The measurement ranges of these assays were 0.5–850 KIU/ml, 5–5,000 KIU/ml, and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with PBS and reanalyzed. All values are reported as log IU/ml.

ISDR and Core aa Substitutions

Amino acid substitutions in the HVC core and ISDR regions were determined by direct sequencing of PCR products following extraction and reverse transcription of HCV RNA using serum samples kept frozen at −80°C. Core amino acid substitutions at positions 70 and 91 (core70 and core91, respectively) were determined according to Akuta et al. [2007, 2006], and the number of ISDR substitutions was established as in Enomoto et al. [1995b, 1996].

SNP Genotyping

Each patient was genotyped for two IL28B SNPs previously reported to be associated with therapy outcome: rs12979860 and rs8099917, and a SNP reported to be associated with ribavirin-induced anemia: rs1127354. Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip, the Invader assay, or the TaqMan assay, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003]. The two SNPs in the IL28B locus are in strong linkage disequilibrium, with a correlation coefficient of 0.99.

Statistical Analysis

The χ^2 and Mann–Whitney U-tests were applied to detect significant associations. Simple and multiple regression analyses were used to examine the association between treatment outcome and the values of other markers, using $P < 0.1$ as the criterion for inclusion in the multivariate model. All of the statistical analyses were two sided, and $P < 0.05$ was considered significant. All statistical analysis was performed using the PASW Statistics 18 program (SPSS, Inc., Chicago, IL).

RESULTS

Reduction of Hemoglobin Levels During Therapy by ITPA Genotype

Decrease in hemoglobin levels during therapy was analyzed by rs1127354 genotype (CC vs. non-CC). As shown in Figure 1, a rapid decrease in hemoglobin levels during the initial 4 weeks was observed in genotype CC patients. Hemoglobin levels in genotype

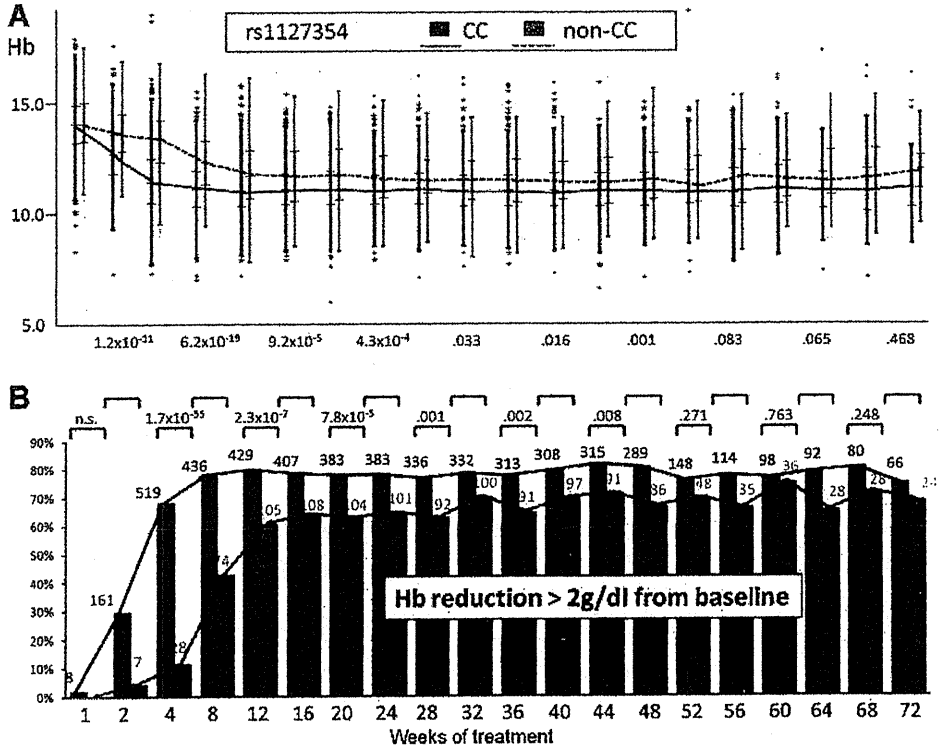

Fig. 1. Reduction of hemoglobin levels by ITPA polymorphism during peg-interferon plus ribavirin combination therapy. **A**: Hemoglobin levels in patients who were treated during the course of therapy. Patients were grouped by ITPA SNP rs1127354 genotype (CC or non-CC). Follow-up hemoglobin levels following cessation of therapy are not shown. **B**: Number of patients who showed >2 g/dl of hemoglobin. Statistical significance was assessed using the χ^2 and Mann–Whitney *U*-tests.

CC patients stabilized by week 8 and did not decrease further. In contrast, a slow but continuous decrease in hemoglobin level was observed in non-CC patients until week 48 (Fig. 1A). Reduction of hemoglobin by more than 2 g/dl was observed significantly more frequently in CC genotype patients than in non-CC patients (Fig. 1B). Differences between the two groups of patients were most pronounced between weeks 2 and 8 (Fig. 1B).

Ribavirin Dose Reduction by ITPA Genotype and Pretreatment Hemoglobin Levels

Decrease in hemoglobin levels resulted in ribavirin dose reduction. The frequency of hemoglobin decrease was higher in genotype CC patients compared with non-CC patients (Fig. 2A). Based on the assumption that initial hemoglobin levels influence incidence of ribavirin dose reduction, reduction frequency was analyzed by initial hemoglobin levels. As shown in Figure 2B–D, reduction of ribavirin was more frequent in genotype CC patients than non-CC patients in all three subsets of patients but was more prominent in patients with intermediate pretreatment hemoglobin levels between 13.5 and 15 g/dl (Fig. 2B–D).

Effect of ITPA Genotype and Pretreatment Hemoglobin Levels on Patients Receiving at Least 80% of Planned Ribavirin Administration

The reduction of ribavirin dosage during therapy resulted in reduction of the total amount of ribavirin given to each patient. As 80% of planned ribavirin administration appears to be a threshold associated with treatment outcome in patients with genotype 1b [McHutchison et al., 2002], the proportion of patients who received more than 80% of the initially planned dosage of ribavirin in 830 patients with genotype 1 and treated with the combination therapy (Table I) were analyzed. As shown in Figure 3, patients with non-CC genotypes tended to tolerate more than 80% of the predetermined dose of ribavirin compared with patients with CC. The difference was statistically significant only in patients whose pretreatment hemoglobin level was 13.5–15 g/dl, however (Fig. 3).

Factors Associated With Successful Administration of at Least 80% of Planned Ribavirin Dose

As it is possible that several factors including ITPA genotype and pretreatment hemoglobin levels are associated with dose reduction of ribavirin, the

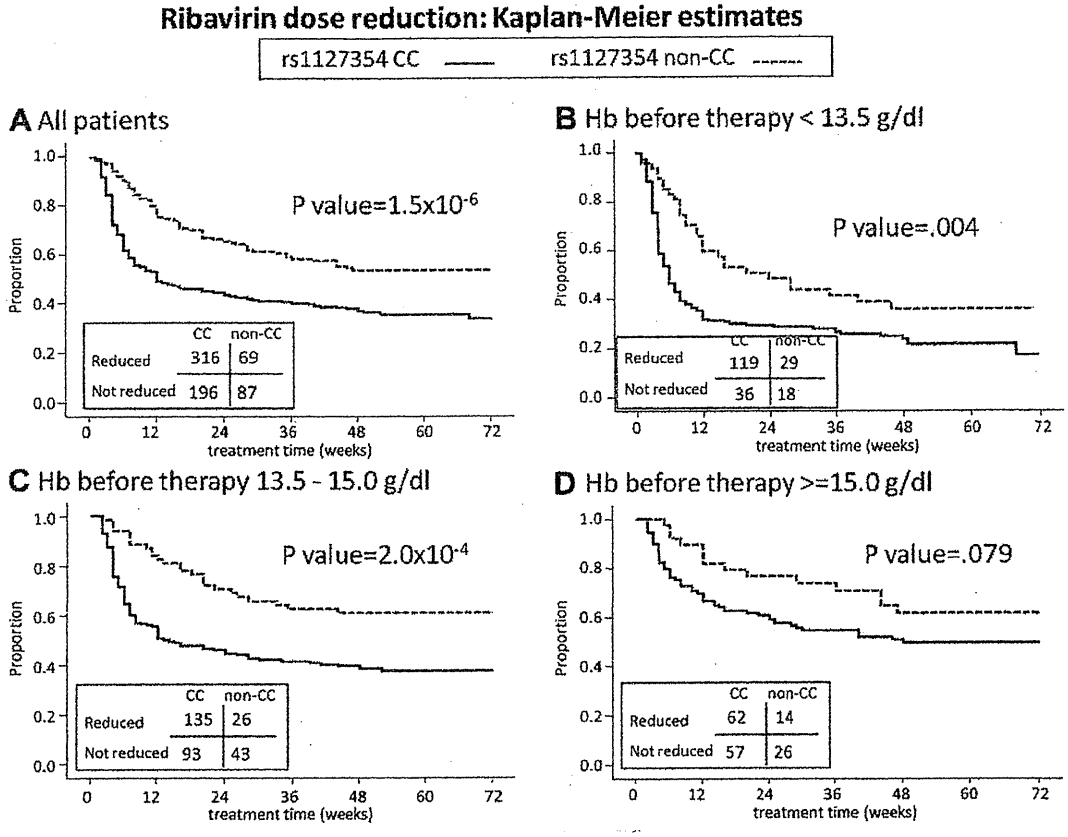

Fig. 2. Dose reduction of ribavirin in patients who were treated with combination therapy. Kaplan–Meier curves for dose reduction grouped by ITPA SNP rs1127354 genotype (solid line: CC, dashed-line: non-CC) among (**A**) all patients, (**B**) patients with low pretreatment hemoglobin levels (<13.5 g/dl), (**C**) patients with intermediate pretreatment hemoglobin levels (13.5–15.0 g/dl), and (**D**) patients with high pretreatment hemoglobin levels (≥15 g/dl).

effect of these factors as well as clinical factors were analyzed for dose reduction of ribavirin. As shown in Table II, univariate analysis identified ITPA SNP rs1127354 genotype, fibrosis stage and inflammatory activity of the liver, white blood cell count, platelet count, hemoglobin, ALT, age, and sex as factors associated with more than 80% ribavirin administration. Multivariate analysis identified age, hemoglobin, and rs1127354 genotype as independent predictive factors.

Effect of ITPA Genotype and Pretreatment Hemoglobin Levels on Outcome of Therapy

As the frequency of patients receiving more than 80% of planned ribavirin administration differed by pretreatment hemoglobin levels and ITPA genotype, treatment outcome might be expected to differ based on these factors. As expected, SVR rate was significantly higher in patients with non-CC genotypes with hemoglobin levels 13.5–15 g/dl, where the frequency of patients receiving 80% ribavirin administration differed most significantly between genotypes CC and non-CC (Fig. 4).

Predictive Factors of the Combination Therapy for SVR and NVR

Predictive factors for SVR and NVR were assessed, including baseline clinical factors, genotype of the recently reported IL28B SNP, and viral factors such as the number of substitutions in the ISDR, and substitutions at core amino acid 70 and 91. By univariate analysis, a number of factors were significantly associated with SVR, including IL28B SNP genotypes (rs8099917 and rs12979860), ITPA SNP rs1127354 genotype, core70 mutation, fibrosis of the liver, white blood cell count, platelet count, hemoglobin, ALT, fasting blood sugar, viral titer, age, sex, body mass index, and duration of the therapy (Table III). Multivariate analysis identified IL28B SNP rs8099917 genotype as the strongest independent predictor for SVR (OR 15.379, $P = 3.48E{-}07$), followed by hemoglobin level, ITPA SNP rs1127354 genotype, fibrosis of the liver, age, and body mass index (Table III). Significant independent predictive factors for NVR included IL28B SNP rs8099917 genotype fibrosis, and age (Table IV).

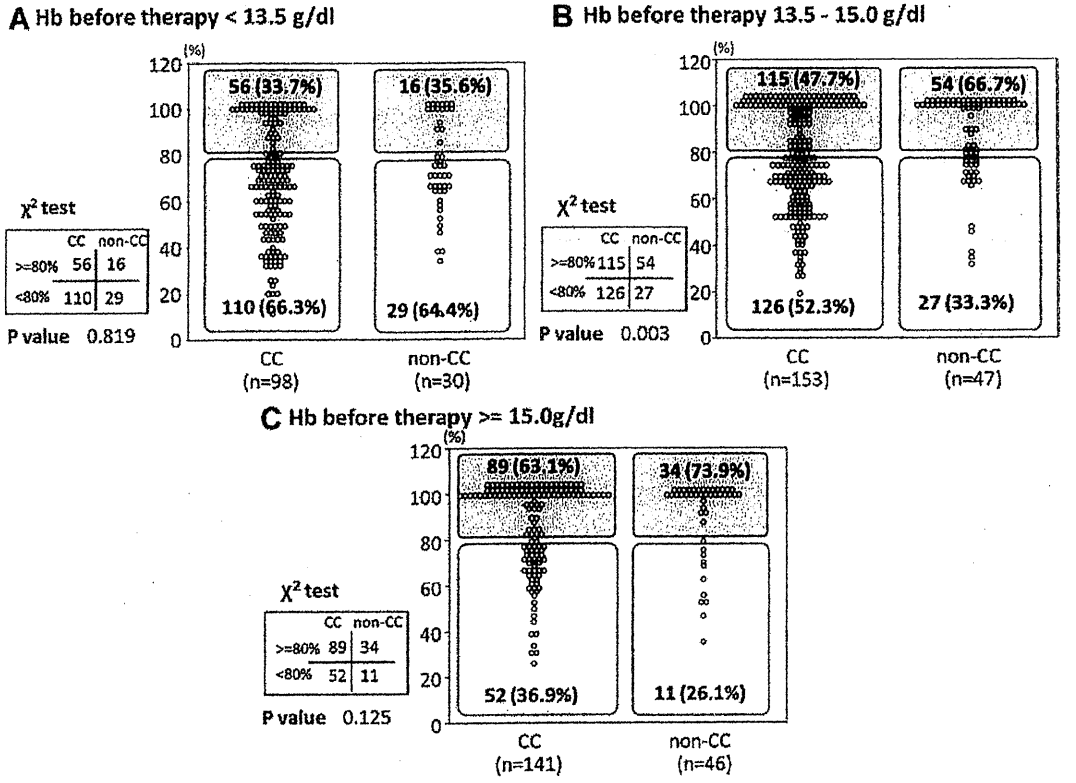

Fig. 3. Dose of ribavirin administered to patients with genotype 1 treated with combination therapy by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (**A**) <13.5 g/dl, (**B**) between 13.5 and 15.0 g/dl, and (**C**) ≥15 g/dl.

TABLE II. Factors Associated With Ribavirin Dose Reduction (80%) in Hepatitis C Virus Patients Determined by Logistic Regression Analysis

	Simple		Multiple		
Variable	OR	*P*-value	OR	95% CI	*P*-value
rs1127354 CC vs. CA/AA	0.580	0.002**	0.578	0.372–0.897	0.014*
Core70	1.007	0.974			
Core91	0.776	0.244			
ISDR 0/1 vs. >1	1.091	0.743			
BMI (kg/m^2)	1.008	0.740			
Fibrosis 1–2 vs. 3–4	1.676	0.009**	1.409	0.902–2.202	0.132
Activity 0–1 vs. 2–3	1.537	0.013*			
WBC (/mm^3)	1.000	1.2E−05**			
Plt (×10^4/mm^3)	1.070	5.2E−06**	1.000	1.000–1.000	0.178
Hb (g/dl)	1.485	1.7E−10**	1.244	1.066–1.453	0.006**
AST (IU/L)	1.001	0.769			
ALT (IU/L)	1.003	0.035*			
γGTP (IU/L)	1.001	0.362			
Albumin (g/dl)	1.549	0.460			
Total cholesterol (mg/dl)	0.997	0.175			
Triglycerides (mg/dl)	1.000	0.935			
HDL cholesterol (mg/dl)	0.989	0.066			
LDL cholesterol (mg/dl)	0.995	0.503			
Fasting blood sugar (mg/dl)	1.001	0.585			
Virus titer (log IU/ml)	1.047	0.567			
Age	0.936	2.1E−15**	0.934	0.914–0.954	3.5E−10**
Sex	0.586	3.9E−04**			

**$P < 0.01$.
*$P < 0.05$.

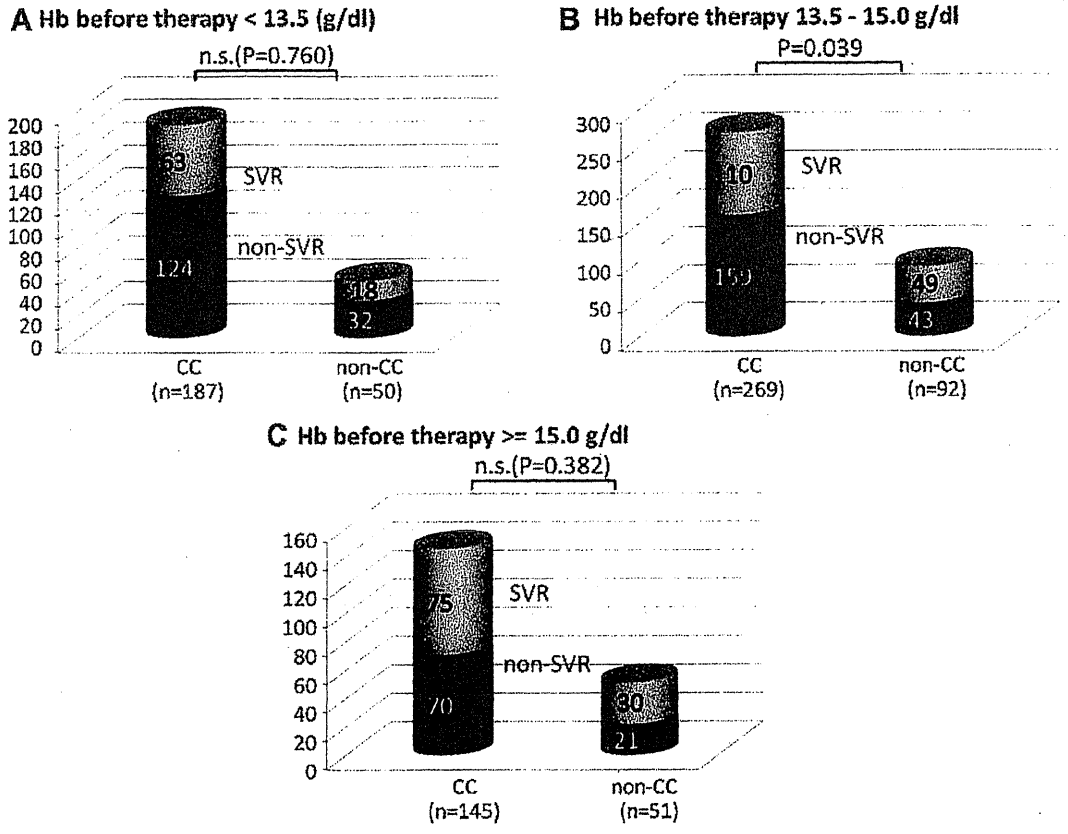

Fig. 4. Effect of combination therapy in patients with genotype 1b by ITPA rs1127354 genotype and pretreatment hemoglobin levels. Patients with genotype 1b and treated with ribavirin were divided into three groups based on their pretreatment hemoglobin levels: (**A**) <13.5 g/dl, (**B**) between 13.5 and 15.0 g/dl, and (**C**) ≥15 g/dl.

DISCUSSION

Ribavirin-induced anemia is one of the most serious side effects resulting from combination therapy [De Franceschi et al., 2000], but a polymorphism within the ITPA gene has recently been shown to affect incidence of this form of anemia [Fellay et al., 2010; Ochi et al., 2010; Thompson et al., 2010]. This study showed that hemoglobin decrease is faster and more severe, especially in the first 12 weeks of treatment, in patients with the anemia-susceptible ITPA rs1127354 CC genotype (Fig. 1). The rapid reduction of hemoglobin observed in genotype CC patients persisted to the end of therapy and was associated with early reduction of ribavirin dosage (Fig. 2), resulting in lower total ribavirin administration. The linear and continuous decrease in hemoglobin seen in non-CC patients also contributed to the reduction of ribavirin administration but not as drastically as in patients with the CC genotype (Fig. 2). The other significant ITPA SNP, rs7270101, is associated with splicing variant formation and reduced activity of the ITPA enzyme in patients of European and African ancestry, but this SNP is absent in the Japanese population [Ochi et al., 2010]. Therefore, only the missense SNP rs1127354, which results in a P32T amino acid change and reduced enzyme activity, was analyzed. Thompson et al. [2010] divided patients into four groups (−, +, ++, +++) based on the genotypes of these two SNPs. According to their classification, CC and non-CC genotypes in this study are almost comparable to "−" and "++" in their study because there are no patients with the rs1127354 AA genotype, and there were only two "+++" patients present in their study. Hemoglobin decrease was slightly milder in the this study compared to Thompson et al. [2010], probably due to early reduction in ribavirin dose in Japanese patients resulting from lower pretreatment hemoglobin levels.

Initial hemoglobin levels indeed had a strong influence on reduction of ribavirin dose. As shown in Figure 3, ITPA genotype did not have a significant influence on patients with <80% ribavirin administration when pretreatment hemoglobin levels were <13.5 or >15 g/dl. Accordingly, because reduction of ribavirin to <80% results in decreased rate of SVR [McHutchison et al., 2002], patients with pretreatment hemoglobin levels below 13.5 g/dl or patients with pretreatment hemoglobin levels between 13.5 and 15 g/dl who have the ITPA anemia-susceptible genotype should receive treatment with drugs such as erythropoietin to prevent reduction of ribavirin.