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A Pilot Study of Triple Therapy With Telaprevir, Peginterferon and Ribavirin for Elderly Patients With Genotype 1 Chronic Hepatitis C

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The prevalence of hepatitis C virus (HCV) infection in elderly patients has been increasing in Japan. However, there are no reports on the safety and efficacy of the triple therapy of telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection. This study evaluated the safety and efficacy of triple therapy [12 weeks of telaprevir 1,500 mg/day, reduction dose, and 24 weeks of peginterferon and ribavirin] in 18 elderly Japanese patients aged >65 years, with chronic infection with HCV genotype 1b. Four patients received triple therapy with telaprevir 2,250 mg/day and the other 14 patients received telaprevir 1,500 mg/day. Sustained virological response-12 (HCV RNA negativity at 12 weeks after completion of therapy) was 50% (9 of 18 patients); while 4 of 18 (22%) patients discontinued triple therapy due to adverse events (skin rashes, anemia, poor appetite). The dose of telaprevir did not affect HCV RNA clearance rates. Regardless of the dose, 50% of the treated patients achieved sustained virological response-12, evaluated by intention-to-treat analysis. Furthermore, the fall in hemoglobin and the rise in serum creatinine were significantly milder in the telaprevir 1,500 mg group than the telaprevir 2,250 mg/day group. Further analysis showed that 67% (6 of 9 elderly patients) with IL28B gene (rs8099917) genotype TT, treated with telaprevir 1,500 mg, achieved sustained virological response-12. These results suggest that 24-week triple therapy with telaprevir 1,500 mg seems safe and efficacious for elderly Japanese patients infected with HCV genotype 1b. ***J. Med. Virol. 85:1746–1753, 2013.*** © 2013 Wiley Periodicals, Inc.

KEY WORDS: HCV; telaprevir; peginterferon; ribavirin; elderly patient

INTRODUCTION

Hepatitis C virus (HCV) often causes chronic liver infection, and can potentially cause liver cirrhosis and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. There is a growing need for treatment of chronic HCV in elderly patients with increased proportion of such patients in the last few decades. This is important since Japanese patients infected with HCV are much older than Western patients due to the widespread HCV infection that affected Japan about 20 years ago [Yoshizawa et al., 2006].

Sustained virological responders who are negative for serum HCV RNA at 24 weeks after the completion of interferon therapy are likely to remain in virological and biochemical remission and show histological improvement [Marcellin et al., 1997; Shiratori et al., 2000]. In addition, interferon therapy reduces the risk of HCC in virological or biochemical responders [Imai et al., 1998; Ikeda et al., 1999; Yoshida et al., 1999]. Especially, HCV in elderly patients is associated with hepatocarcinogenesis and poor survival [Ikeda et al., 2009], and sustained virological response to interferon therapy is associated with improved clinical outcome [Asahina et al., 2010]. However, the sustained virological response rate tends to be lower in elderly patients with chronic hepatitis C, due in part to less tolerability and efficacy of interferon (IFN) combination therapy compared with adult patients [Iwasaki et al., 2006; Honda et al., 2010].

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Several direct acting antiviral agents have been designed and developed recently, represented by NS3/4A or NS5A protease inhibitors and NS5B polymerase or NS5A inhibitors [Asselah and Marcellin, 2011]. Among them, telaprevir has shown more effective results when combined with peginterferon and ribavirin in the treatment of chronic hepatitis C than peginterferon and ribavirin combination therapy [McHutchison et al., 2009, 2010; Hézode et al., 2010; Kumada et al., 2011]. However, there are no reports about the safety and efficacy of the triple therapy, which are combined with telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection. Clinically, it is important to determine whether elderly patients with HCV infection can be treated with triple therapy of telaprevir, peginterferon, and ribavirin.

The aim of this pilot study was to evaluate the safety and efficacy of triple therapy with telaprevir, peginterferon, and ribavirin for elderly patients with chronic HCV infection genotype 1b.

PATIENTS AND METHODS

Study Population

From May 2008 through November 2012, 297 patients with chronic hepatitis C were selected for treatment with telaprevir, peginterferon, and ribavirin at the Department of Hepatology, Toranomon Hospital (located in metropolitan Tokyo). Subsequently, 18 of these patients received the triple therapy based on the following inclusion criteria: (1) diagnosis of chronic HCV infection; (2) infection with HCV genotype 1b confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels >5.0 $\log_{10}$ IU/ml, determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan); (4) Japanese aged ≥66 years at the start of treatment; (5) agreed to be treated with telaprevir, peginterferon, and ribavirin; (6) no evidence of liver cirrhosis; (7) no evidence of HCC; (8) negative for hepatitis B surface antigen; (9) no evidence of human immunodeficiency virus infection; (10) no evidence of autoimmune hepatitis, alcoholic liver disease, hemochromatosis or chronic liver disease other than chronic HCV infection; and (11) no history of cardiac disease, cerebral disorder, and pulmonary disease. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave an informed consent before participating in this trial.

Table I summarizes the profiles and laboratory data of the 18 patients at the time of commencement of treatment. Treatment efficacy was evaluated by intention-to-treat analysis classified as treatment failure in patients who could not complete the treatment regimen. HCV RNA levels and hemoglobin were monitored at baseline and weeks 1, 2, 4, 8, 12, 16, 20, and 24 during treatment.

Four patients were treated with telaprevir 750 mg every 8-hr (q8h) (2,250 mg/day group), while the other 14 patients were treated with telaprevir 750 mg twice daily at 12-hr interval (q12h) (1,500 mg/day group). Peginterferon-α-2b was injected subcutaneously at a median dose of 1.5 μg/kg

TABLE I. Characteristics of Patients at Baseline

Number of patients	18
Age (years)*	68 (66–73)
Male/female	10/8
Body mass index (kg/m^2)*	22.8 (18.9–26.3)
Viral load of HCV ($\log_{10}$ IU/ml)	6.5 (5.1–7.3)
Serum aspartate aminotransferase (IU/L)	36 (11–95)
Serum alanine aminotransferase (IU/L)	38 (19–80)
Serum albumin (g/dl)	3.8 (3.3–4.1)
Gamma-glutamyl transpeptidase (IU/L)	27 (10–62)
Leukocyte count ($/mm^3$)	4,000 (2,500–7,300)
Hemoglobin (g/dl)	14.0 (12.5–16.1)
Platelet count ($\times 104/mm^3$)	15.5 (9.6–21.4)
Alpha-fetoprotein (μg/L)	4 (1–18)
Treatment	
Peginterferon α-2b dose (μg/kg)*	1.5 (1.0–1.8)
Ribavirin dose (mg/kg)*	7.7 (5.8–13.2)
Telaprevir dose(1,500/2,250 mg/day)	14/4
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine/glutamine)	10/8
Core aa 91 (leucine/methionine)	11/7
ISDR of NS5A (wild-type/non wild-type/ND)	17/0/1
Genetic variation near IL28B gene rs8099917 genotype (TT/TG/GG)	11/6/1
Past history of interferon therapy Treatment-naïve/relapsers to previous treatment/nonresponders to previous treatment	3/10/5
Comorbidities[a]	
Diabetes mellitus	3 (17%)
Hypertension	9 (50%)

Data are numbers (percentages) of patients, except those denoted by *, which represent the median (range) values.
[a]All patients were not on medications.

(range: 1.1–1.8 μg/kg) once a week. Ribavirin was administered at a median dose of 8.3 mg/kg body weight (range: 5.8–13.2 mg/kg) twice a day every 12 hr. Each drug was discontinued or its dose reduced, as required upon judgment of the attending physician, in response to a fall in hemoglobin level, leukocyte count, neutrophil count or platelet count, or the appearance of side effects. The triple therapy was discontinued when the leukocyte count decreased to $<1{,}000/mm^3$, neutrophil count $<500/mm^3$, or platelet count $<5 \times 10^4/mm^3$, or when hemoglobin decreased to <8.5 g/dl.

Measurement of HCV RNA

The virological response was assessed using the COBAS TaqMan HCV test. The linear dynamics range of this assay is 1.2–7.8 $\log_{10}$ IU/ml and samples with undetectable HCV RNA were defined as negative. The response to treatment was divided into the following: sustained virological response-12 (negative HCV RNA at 12 weeks after completion of therapy), which is relevant to sustained virological response-24 defined by Martinot-Peignoux et al. [2010] and Mauss et al. [2012], relapse (rise in viral load after the end of treatment, even when HCV RNA was negative at the end of treatment), and viral breakthrough (rise in viral load before the end of treatment, even when HCV RNA was temporarily negative during treatment).

Detection of Amino Acid Substitutions in Core and NS5A Regions of HCV-1b

With the use of HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 amino acids (aa) in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed in a previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. The sequence of 2,209–2,248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [1996] was determined and the numbers of aa substitutions in the ISDR were defined as wild-type (0, 1) or non wild-type (≥2), compared with HCV-J. In the present study, aa substitutions of the core region and NS5A-ISDR of HCV-1b were analyzed by direct sequencing.

Determination of IL-28B Genotype

IL-28B (rs8099917) was genotyped by the Invader assay, Taq Manassay, or direct sequencing, as described previously [Ohnishi et al., 2001; Suzuki et al., 2003].

Statistical Analysis

The χ^2 test, Fisher's exact probability test, and Mann–Whitney's *U*-test were used to compare the background characteristics of the groups. All *P* values were two-tailed, and $P < 0.05$ was considered statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL).

RESULTS

Efficacy of Triple Therapy

Figure 1a illustrates the negative rates of HCV RNA at different time points. The disappearance rate of HCV RNA during treatment was 11% (2/18), 33% (6/18), 83% (15/18), 89% (16/18), 78% (14/18), 78% (14/18), 67% (12/18), and 67% (12/18) at 1, 2, 4, 8, 12, 16, 20, and 24 weeks, respectively. Furthermore, 50% (9/18) of elderly patients achieved sustained virological response-12.

Four of the 18 patients discontinued triple therapy because of side effects, but in the remaining 14 patients, HCV RNA level was below the detection limit of the test during treatment. Two patients experienced viral breakthrough at 20 weeks after the commencement of treatment and three patients experienced relapse. Four patients discontinued the triple therapy due to the appearance of side effects [two developed skin disease (at 4th and 10th week), one developed anemia (at second week), and one patient discontinued due to poor appetite (at the 11th week)]. Figure 1b shows changes in hemoglobin level in patients who received the triple therapy. During the administration of telaprevir to 12 weeks, hemoglobin decreased steadily, with a maximum of 3.7 g/dl (mean value) at 12 week. However, hemoglobin tended to increase after the end of telaprevir medication, during treatment with peginterferon and ribavirin. Figure 1c shows changes in serum creatinine level in patients who received the triple therapy. During administration of telaprevir to 12 weeks, creatinine increased steadily, with a maximum of 0.14 g/dl (mean value) at 8 week. Similar to the pattern described above for hemoglobin, serum creatinine tended to decrease after the end of telaprevir medication, during treatment with peginterferon and ribavirin.

Response to Treatment as a Function of Telaprevir Dose

Table II summarizes the profiles and laboratory data of the 18 patients according to the dose of telaprevir. At baseline, leukocyte count in patients treated with telaprevir 1,500 mg/day was lower than in those treated with 2,250 mg/day. None of the female patients received telaprevir at 2,250 mg/day. The HCV RNA clearance rate was similar in the 2,250 and 1,500 mg/day groups (Fig. 2a). Both doses of telaprevir resulted in fall in hemoglobin, but the falls in the 2,250 mg/day group at 2, 4, 8, weeks after the start of treatment were significantly more profound compared with the 1,500 mg/day group (Fig. 2b). Furthermore, both doses of telaprevir

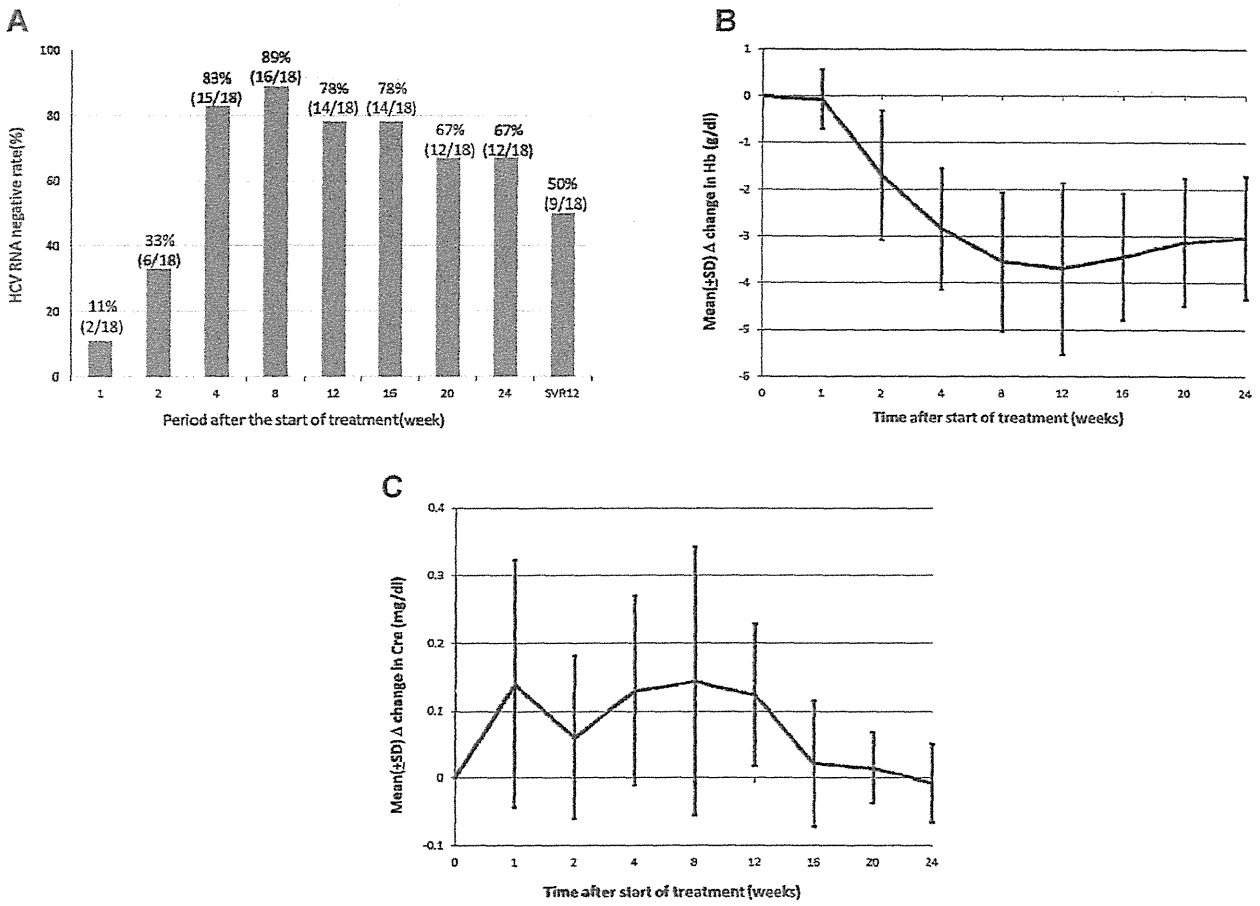

Fig. 1. **a**: HCV RNA clearance rate at different time points after the start of triple therapy of telaprevir with peginterferon and ribavirin. The sustained virological response-12 rate was 50% and the end-of-treatment response rate was 67%. **b**: Fall in hemoglobin in patients who received triple therapy of telaprevir, with peginterferon and ribavirin. **c**: Rise in creatinine in patients who received triple therapy of telaprevir, with peginterferon and ribavirin.

induced a rise in serum creatinine, but the rises in the 2,250 mg/day group at 12, 16, 24, weeks after the start of treatment were significantly more profound compared with the 1,500 mg/day group (Fig. 2c).

Relation between Loss of HCV RNA and IL-28B (rs8099917) Genotype TT

Figure 3a illustrates the negative rates of HCV RNA in patients with the rs8099917 genotype TT/non TT at different time points. The HCV RNA disappearance rate in patients with the rs8099917 genotype TT during treatment was 9% (1/11), 36% (4/11), 82% (9/11), 100% (11/11), 91% (10/11), 91% (10/11), 73% (8/11), and 73% (8/11) at 1, 2, 4, 8, 12, 16, 20, and 24 weeks, respectively. Furthermore, 64% (7/11) of the elderly patients achieved sustained virological response-12.

Figure 3b illustrates the HCV RNA clearance rates in patients with the rs8099917 genotype TT/non TT during treatment with telaprevir 1,500 mg. The HCV RNA clearance rates in patients with the rs8099917 genotype TT during treatment was 11% (1/9), 44% (4/9), 89% (8/9), 100% (9/9), 100% (9/9), 100% (9/9), 78% (7/9), and 78% (7/9) at 1, 2, 4, 8, 12, 16, 20, and 24 weeks, respectively. Furthermore, 67% (6/9) of the elderly patients achieved sustained virological response-12. These results highlight the safety and efficacy of telaprevir 1,500 mg, peginterferon, and ribavirin in elderly patients with the rs8099917 genotype TT.

DISCUSSION

With the aging society in Japan, it is important to evaluate the efficacy of interferon therapy in elderly patients with chronic HCV infection. This is important especially due to the lack of information on the safety and efficacy of triple therapy of telaprevir, peginterferon, and ribavirin. In the study of Suzuki

TABLE II. Characteristics of Patients at Baseline According to Telaprevir Dose and Adherence to Each Drug

Characteristics	Telaprevir dose		*P*-value
	2,250 mg	1,500 mg	
Number of patients	4	14	
Age (years)*	67 (66–68)	69 (66–73)	0.079
Male/female	4/0	10/4	0.023
Body mass index (kg/m^2)*	23.1 (22.3–24.1)	22.6 (18.9–26.3)	NS
Viral load of HCV (log_{10} IU/ml)	5.9 (5.3–7.0)	6.5 (5.1–7.3)	NS
Serum aspartate aminotransferase (IU/L)	43 (37–48)	27 (11–95)	NS
Serum alanine aminotransferase (IU/L)	36 (23–44)	36 (19–80)	NS
Serum albumin (g/dl)	3.9 (3.6–4.0)	3.7 (3.3–4.1)	NS
Gamma-glutamyl transpeptidase (IU/L)	31 (19–62)	22 (10–61)	NS
Leukocyte count ($/mm^3$)	5,400 (4,000–7,300)	3,900 (2,500–5,300)	0.035
Hemoglobin (g/dl)	14.4 (13.5–16.1)	13.9 (12.5–14.9)	NS
Platelet count ($\times 10^4/mm^3$)	16.9 (15.1–21.0)	14.9 (9.6–21.4)	NS
Alpha-fetoprotein (μg/L)	6 (5–7)	3 (1–18)	NS
Treatment			
Peginterferon α-2b dose (μg/kg)*	1.4 (1.3–1.6)	1.5 (1.0–1.8)	
Ribavirin dose (mg/kg)*	12.4 (11.6–13.2)	7.0 (5.8–12.9)	0.005
Amino acid substitutions in the HCV genotype 1b			NS
Core aa 70 (arginine/glutamine)	1/3	9/5	NS
Core aa 91 (leucine/methionine)	2/2	9/5	NS
ISDR of NS5A (wild-type/non wild-type/ND)	4/0/0	13/0/1	NS
Genetic variation near IL28B gene rs8099917 genotype (TT/TG/GG)	2/1/1	9/5/0	NS
Past history of interferon therapy Treatment-naïve/relapsers to previous treatment/nonresponders to previous treatment	2/2/0	1/8/5	0.087
PegIFN adherence (%)	78.7 (55.6–100)	80.0 (8.3–100)	NS
RBV adherence (%)	33.9 (17.7–68.8)	50.0 (6.7–79.2)	NS
TVR adherence (%)	68.4 (36.7–100)	66.7 (11.2–66.7)	NS
Comorbidities[a]			
Diabetes mellitus	1 (25%)	2 (14%)	NS
Hypertension	2 (50%)	7 (50%)	NS

Date are number (percentage) of patients, except those denoted by *, which represent the median (range) values.
[a]All patients were not on medications.

et al. [2012], 20 patients with chronic HCV infection and high viral load of genotype 1b were randomly assigned to two telaprevir-based regimens of 2,250 and 1,500 mg/day in combination with peginterferon and ribavirin for 12 weeks. The sustained virological response rates were not different between the 1,500 and 2,250 mg groups, while serum creatinine increased more extensively in the 2,250 mg group than in the 1,500 mg group. However, their patients were <65 years old and treated for only 12 weeks. In the present study, the response to triple therapy with telaprevir for 12 weeks, peginterferon, and ribavirin for 24 weeks was examined in a pilot study that included 18 elderly patients infected with HCV-1b with high viral loads. Four of the 18 patients were treated with telaprevir 2,250 mg/day and the other 14 patients were treated with telaprevir 1,500 mg/day. The results showed no tolerance to the triple therapy in 4 of 18 (22%) patients due to skin rashes, anemia, and poor appetite. However, 9 of 18 (50%) elderly patients who received the triple therapy were able to achieve sustained virological response-12. Furthermore, even when treated for 24 weeks, elderly patients of the 1,500 mg group showed reduction in the elevated serum creatinine that was similar to that seen in patients aged <65 years.

The IL-28B genotype is identified as a pretreatment predictor of virological response to 48-week peginterferon plus ribavirin combination therapy in individuals infected with HCV-1 [Ge et al., 2009; Tanaka et al., 2009; Suppiah et al., 2009], and also as a predictor of response to triple therapy with telaprevir, peginterferon, and ribavirin in Japanese patients infected with HCV-1 [Akuta et al., 2010, 2012; Chayama et al., 2011]. In the present study, among patients with the rs8099917 genotype TT who were treated with telaprevir 1,500 mg, 6 of 9 (67%) could achieve sustained virological response-12, and none discontinued the triple therapy because of side effects. Thus, for elderly patients with the rs8099917 genotype TT, triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin was safe and efficacious, especially in patients with the rs8099917 genotype TT.

Iwasaki et al. [2006] and Honda et al. [2010] reported that the sustained virological response rates at the completion of the 48-week interferon and ribavirin combination therapy for elderly patients were only 16% and 31%, respectively. However, in the present study, of 18 elderly patients, 12 (67%) were negative for HCV RNA at the end of the triple therapy, and sustained virological response-12 was

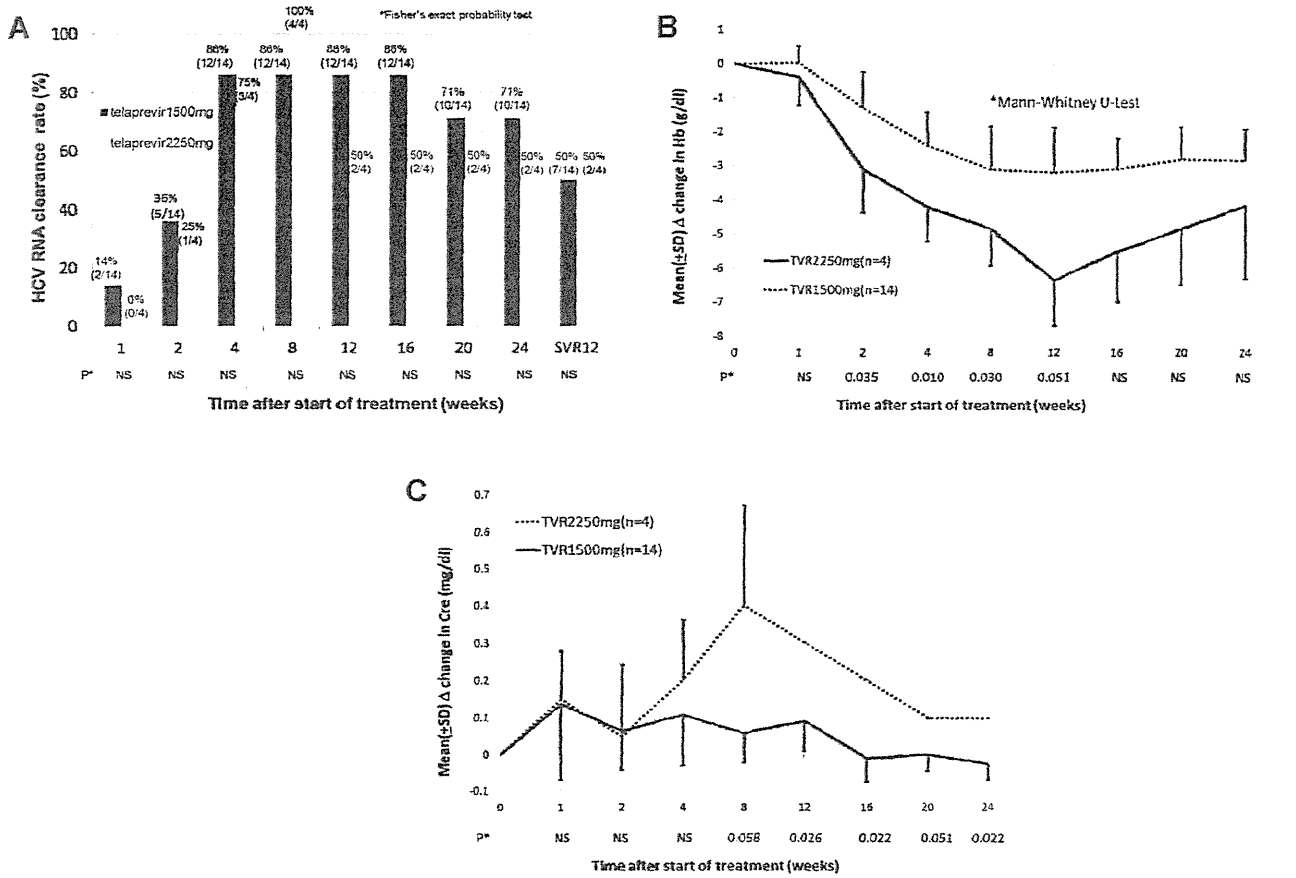

Fig. 2. **a**: HCV RNA clearance rate according to the dose of telaprevir (1,500 and 2,250 mg/day), combined with peginterferon and ribavirin. The sustained virological response-12 rate was 50% in both dose groups and the end-of-treatment response rates were 71% and 50%, respectively. **b**: Fall in hemoglobin according to the dose of telaprevir (1,500 and 2,250 mg/day), in combination with peginterferon and ribavirin. The fall was more profound in the 2,250 mg/day group at 2, 4, and 8 weeks compared with the 1,500 mg/day group. **c**: Rise in serum creatinine according to the dose of telaprevir (1,500 and 2,250 mg/day), in combination with peginterferon and ribavirin. The rise was more profound in the 2,250 mg/day group at 12, 16, and 24 weeks compared with the 1,500 mg/day group.

achieved by 9 patients (50%). Analysis of the data of the 14 elderly patients showed sustained virological response-12 was achieved in seven (50%) patients who received triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin, seven (50%). These results indicate that triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin, is safe and efficacious. Further studies are needed to determine if such treatment can be shortened to 24 weeks.

This study is not without limitations. The number of patients who received triple therapy was small and the study failed to show statistical significance in any comparison of various factors, especially between telaprevir 1,500 mg and telaprevir 2,250 mg treatment groups. This study is retrospective in nature; therefore, selection bias may have affected the results. We did not estimate sustained virological response-24 in the present study. Martinot-Peignoux et al. [2010] and Mauss et al. [2012] reported sustained virological response-12 as endpoint for future trials because HCV relapse usually occurs within the first 12 weeks after the end of treatment. Accordingly, in this study, we estimated sustained virological response-12. To generalize medical treatment for elderly patients with chronic HCV infection, further large scale randomized control clinical trials for telaprevir 1,500 mg and 2,250 mg are necessary to investigate the sustained virological response-24.

In conclusion, triple therapy with telaprevir 1,500 mg, peginterferon, and ribavirin, is safe and efficacious in elderly patients with chronic HCV infection. The triple therapy could be selected as

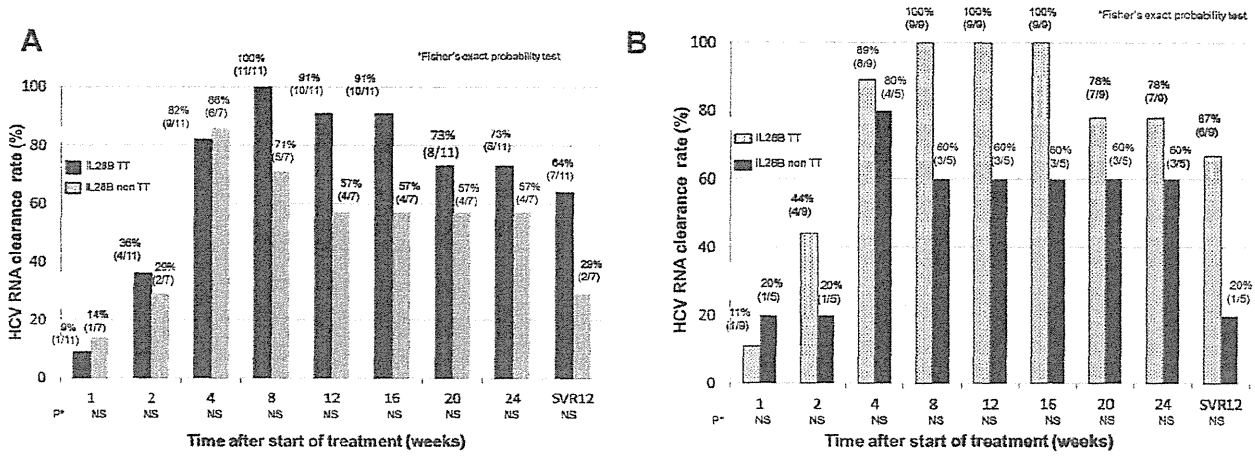

Fig. 3. **a**: HCV RNA clearance rate according to rs8099917 genotype TT. The sustained virological response-12 rate was 64% and the end-of-treatment response rate was 73% in patients with rs8099917 genotype TT. **b**: HCV RNA clearance rate according to rs8099917 genotype TT after the start of triple therapy of telaprevir 1,500 mg with peginterferon and ribavirin. The sustained virological response-12 rate was 78% and the end-of-treatment response rate was 67%.

potentially suitable therapy for elderly Japanese patients aged >66 years with chronic HCV of genotype 1b.

REFERENCES

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. 2010. Amino acid substitution in HCV core region and genetic variation near IL28B gene predict viral response to telaprevir with peginterferon and ribavirin. Hepatology 52:421–429.

Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Chayama K, Nakamura Y, Kumada H. 2012. Amino acid substitution in HCV core region and genetic variation near the IL28B gene affect viral dynamics during telaprevir, peginterferon and ribavirin treatment. Intervirology 55:417–425.

Asahina Y, Tsuchiya K, Tamaki N, Hirayama I, Tanaka T, Sato M, Yasui Y, Hosokawa T, Ueda K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Enomoto N, Izumi N. 2010. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. Hepatology 52:518–527.

Asselah T, Marcellin P. 2011. New direct-acting antivirals' combination for the treatment of chronic hepatitis C. Liver Int 31:S68–S77.

Chayama K, Hayes CN, Abe H, Miki D, Ochi H, Karino Y, Toyota J, Nakamura Y, Kamatani N, Sezaki H, Kobayashi M, Akuta N, Suzuki F, Kumada H. 2011. IL28B but not ITPA polymorphism is predictive of response to pegylated interferon, ribavirin, and telaprevir triple therapy in patients with genotype 1 hepatitis C. J Infect Dis 204:84–93.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. 2009. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461:399–401.

Hézode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourlière M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM, Zeuzem S, PROVE2 Study Team. 2010. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 360:1839–1850.

Honda T, Katano Y, Shimizu J, Ishizu Y, Doizaki M, Hayashi K, Ishigami M, Itoh A, Hirooka Y, Nakano I, Urano F, Yoshioka K, Toyoda H, Kumada T, Goto H. 2010. Efficacy of peginterferon-alpha-2b plus ribavirin in patients aged 65 years and older with chronic hepatitis C. Liver Int 30:527–537.

Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, Tsubota A, Nakamura I, Murashima N, Kumada H, Kawanishi M. 1999. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C; a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 29:1124–1130.

Ikeda K, Arase Y, Kawamura Y, Yatsuji H, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Saitoh S, Suzuki F, Suzuki Y, Kumada H. 2009. Necessities of interferon therapy in elderly patients with chronic hepatitis C. Am J Med 122:479–486.

Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, Ishikawa H, Matsuda Y, Nishikawa M, Seki K, Matsuzawa Y. 1998. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C, Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med 129:94–99.

Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M, Shimoe T, Takaguchi K, Hashimoto N, Kobatake T, Tomita M, Kawaguchi M, Kobashi H, Sakaguchi K, Shiratori Y. 2006. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 43:54–63.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 87:9524–9528.

Kenny-Walsh E. 1999. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med 340:1228–1233.

Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H, Hayashi N. 2011. Telaprevir with peginterferon and ribavirin for treatment-naïve patients chronically infected with HCV of genotype 1 in Japan. J Hepatol 56:78–84.

Marcellin P, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Degott C, Erlinger S. 1997. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. Ann Intern Med 127:875–881.

Martinot-Peignoux M, Stern C, Maylin S, Ripault MP, Boyer N, Leclere L, Castelnau C, Giuily N, El Ray A, Cardoso AC, Moucari R, Asselah T, Marcellin P. 2010. Twelve weeks posttreatment follow-up is as relevant as 24 weeks to determine the sustained virologic response in patients with hepatitis C virus receiving pegylated interferon and ribavirin. Hepatology. 51:1122–1126.

Mauss S, Berg T, Rockstroh J, Sarrazin C, Wedemeyer H. 2012. Hepatology a clinical text book. 3rd edition. Flying Publisher. 202 p.

McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ, PROVE1 Study Team. 2009. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 360:1827–1838.

McHutchison JG, Manns MP, Muir AJ, Terrault NA, Jacobson IM, Afdhal NH, Heathcote EJ, Zeuzem S, Reesink HW, Garg J, Bsharat M, George S, Kauffman RS, Adda N, Di Bisceglie AM, PROVE3 Study Team. 2010. Telaprevir for previously treated chronic HCV infection. N Engl J Med 362:1292–1303.

Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hürter D, Nawrocki M, Kruska L, Hensel F, Petry W, Häussinger D. 1998. Prognosis of chronic hepatitis C: Results of a large, prospective cohort study. Hepatology 28:1687–1695.

Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. 2001. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 46:471–477.

Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M. 2000. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 132:517–524.

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. 2009. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1104.

Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, Nagasaki M, Nakayama-Hamada M, Kawaida R, Ono M, Ohtsuki M, Furukawa H, Yoshino S, Yukioka M, Tohma S, Matsubara T, Wakitani S, Teshima R, Nishioka Y, Sekine A, Iida A, Takahashi A, Tsunoda T, Nakamura Y, Yamamoto K. 2003. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginiedeminase 4, are associated with rheumatoid arthritis. Nat Genet 34:395–402.

Suzuki F, Suzuki Y, Sezaki H, Akuta N, Seko Y, Kawamura Y, Hosaka T, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Mineta R, Watahiki S, Kobayashi M, Nakayasu Y, Tsuda H, Aoki K, Yamada I, Kumada H. 2012. An exploratory study on telaprevir given every 8 hours at 500 or 750 mg with peginterferon alfa-2b and ribavirin in hepatitis C patients. Hepatol Res, in press.

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109.

Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. 1999. Interferon therapy reduces the risk for hepatocellular carcinoma: National surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. Ann Intern Med 131:174–181.

Yoshizawa H, Tanaka J, Miyakawa Y. 2006. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. Intervirology 49:7–17.

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Seroclearance rate of hepatitis B surface antigen in 2,112 patients with chronic hepatitis in Japan during long-term follow-up

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Abstract
Background Rate of hepatitis B surface antigen (HBsAg) seroclearance was determined in 2,112 Japanese patients with chronic hepatitis B who were followed up for at least 15 years.
Methods Patients had a median age of 37 years and included 1,431 (67.8 %) men. Median values were AST/ALT, 43/62 IU/L; platelet counts, 182 × 10^3/mm^3; HBsAg, 3,400 IU/mL; and hepatitis B virus (HBV) DNA, 6.2 log copies/mL. Factors influencing HBsAg seroclearance were evaluated by the Cox proportional model and annual rate of HBsAg seroclearance by the Kaplan–Meier life table method.
Results The overall annual rate of HBsAg seroclearance was 1.75 % in 2,112 patients; it was 1.65 % in 1,130 untreated and 2.05 % in 982 treated patients ($p = 0.289$). In untreated patients, seroclearance was influenced by age, no HBV infections in third-degree or closer relatives, and HBsAg levels in univariate analysis. Seroclearance was influenced by a median age ≥50 years [relative risk (RR) 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] in multivariate analysis. In treated patients, age, male gender, no HBV infections in third-degree or closer relatives, interferon therapy, chronic hepatitis, high AST and γ-GTP levels, low platelet counts, hepatitis B e antigen (HBeAg)-negative status, low HBsAg levels and the wild-type precore sequence significantly influenced HBsAg seroclearance. In multivariate analysis, no family history [RR 2.22 ($p = 0.006$)], interferon treatment [RR 3.15 ($p < 0.001$)], and HBeAg-negative status [RR 3.75 ($p < 0.001$)] significantly influenced HBsAg seroclearance.
Conclusions In this retrospective cohort study, the annual rate of HBsAg seroclearance was 1.65 % in untreated patients and 2.05 % in treated patients.

Keywords Seroclearance · Hepatitis B surface antigen · Hepatitis B virus · Chronic hepatitis B

Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ETV	Entecavir
HBeAg	Hepatitis B e antigen
HBcrAg	Hepatitis B core-related antigen
HBV	Hepatitis B virus
HBV DNA	Hepatitis B virus DNA
HBsAg	Hepatitis B surface antigen
IFN	Interferon
LAM	Lamivudine

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Introduction

Worldwide, an estimated 400 million people are infected with hepatitis B virus (HBV) persistently. HBV infection is a common disease that can induce a chronic carrier state

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and is associated with the risk of developing progressive disease and hepatocellular carcinoma (HCC) [1–5]. In regions highly endemic for HBV, such as Asia and Africa, the persistent carrier state is established by perinatal transmission or early in infancy. Carriers serve as the reservoir of HBV in the community and can spread the infection to susceptible individuals. The incidence of HCC is decreased extremely by eradicating HBV from the circulation that is responsible for liver damage [6–9]. In Japan, interferon (IFN) was introduced for the treatment of persistent HBV infections, and long-term IFN increased seroclearance of hepatitis B surface antigen (HBsAg) [10]. Since 2000, the effect of long-term nucleot(s)ide analogues, such as lamivudine [11, 12] and entecavir [13], on HBsAg seroclearance has been monitored in Japan.

In the current study, we followed untreated or treated patients for at least 15 years. We evaluated the seroclearance of HBsAg, achieved in both groups of patients, by using highly sensitive assays. Our aim was to determine factors that can lead to HBsAg seroclearance and to elucidate the factors associated with its success.

Patients and methods

Patients

During at least 15 years from 1968, 2,112 consecutive patients, chronically mono-infected with HBV (confirmed by HBsAg-positivity for at least 6 months) were followed at the Department of Hepatology, Toranomon Hospital, in Metropolitan Tokyo. Patients met the following inclusion and exclusion criteria: (1) negativity for hepatitis C antibody and/or hepatitis C virus RNA by polymerase chain reaction (PCR) in the serum; (2) no history of HCC; and (3) no history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis B. Thus, the 2,112 patients were enrolled in this cohort study. A written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved a priori by the institution's human research committee.

Treatment

Nine hundred and eighty-two patients received antiviral treatments. Of them, 156 patients received prednisolone (PSL) 40 mg daily for 1 week, 30 mg daily for 1 week, 20 mg daily for 1 week, and then 10 mg daily for 1 week until it was abruptly withdrawn (total 700 mg). A total of 428 patients received 100 mg lamivudine (LAM) daily as an initial therapy. In total, 333 patients received 3–12 MU of IFN-α or IFN-β. The durations and regimens of treatment were as follows: daily for 2 or 4 weeks and then 2 or 3 times per week for 26–104 weeks. The median duration of treatment was 26 weeks (range 4–981). There were 190 (57 %) patients who received multiple treatments of IFN.

LAM treatment was continued as a rule; median duration of LAM treatment was 75 months (55–102). LAM-resistant rtM204I/V mutants developed in 151 (35 %) of the 428 patients, and they were provided with adefovir dipivoxil (10 mg) added on LAM, as a rescue therapy. The remaining patients continued to receive LAM monotherapy. In addition, 65 patients received 0.5 mg entecavir (ETV) daily as an initial therapy. ETV treatment was continued as a rule, and median duration of ETV treatment was 45 months (1.0–104).

Markers of HBV infection

Serum HBsAg titers were determined annually using ARCHITECT HBsAg QT assay kits (Abbott Laboratories, Tokyo, Japan), which have a lower limit of detection of 0.05 IU/mL and an upper limit of detection of 250 IU/mL. To expand the upper limit from 250 to 125,000 IU/mL, serum samples going off the scale were diluted stepwise to 1:20 and 1:500 with ARCHITECT diluents following instructions from the manufacturer.

Hepatitis B e antigen (HBeAg) was determined by enzyme-linked immunosorbent assay with a commercial kit (HBeAg EIA; Institute of Immunology, Tokyo, Japan). HBV DNA was quantified using the Amplicor monitor assay (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.6–7.6 log copies/mL, or COBAS TaqMan HBV v.2.0 (Roche Diagnostics, Tokyo, Japan) with a dynamic range of 2.1–9.0 log copies/mL. Hepatitis B core-related antigen (HBcrAg) was determined by chemiluminescence enzyme immunoassay (CLEIA) with the HBcrAg assay kit (Fujirebio Inc., Tokyo, Japan). A commercial kit (HBV Genotype EIA; Institute of Immunology, Tokyo, Japan) was used to serologically determine HBV genotypes by the combination of epitopes expressed on the pre-S2 region product, which is specific for each of the 7 major genotypes (A–G).

Statistical analysis

Baseline data were obtained on the day of the first visit in untreated patients. In patients who received antivirals, baseline data were obtained at the start of the first day of treatment. Categorical data were compared between groups by chi-squared or Fisher's exact tests. Continuous variables with a nonparametric distribution were analyzed by Mann–Whitney *U* tests, whereas those with a parametric distribution were analyzed by the Student's *t* test. Cox

regression analyses were used to assess variables that were significantly associated with HBsAg seroclearance. All baseline factors that were found to be significantly associated with HBsAg seroclearance by univariate analysis were entered into a multivariate analysis. Independent baseline factors associated with the seroclearance of HBsAg were evaluated using a stepwise Cox regression analysis. We then performed a time-dependent Cox regression to analyze independent factors associated with HBsAg seroclearance while on-treatment factors and independent baseline factors had been adjusted.

Cumulative HBsAg seroclearance rates were analyzed using the Kaplan–Meier method; differences in the resulting curves were evaluated using log-rank tests. Significance was defined as $p < 0.05$ for all two-tailed tests. Data analysis was performed with the SPSS software package version 11.0.1 J (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics in the 2,112 patients

The baseline characteristics of studied patients are shown in Table 1. They had a median age of 37 years (range 1–81), included 1,431 (67.8 %) men, and 2,031 (96.2 %) of them had chronic hepatitis. Their baseline values were AST/ALT, 43 (3–2,192)/62 (2–3,020 IU/L); γ-GTP, 27 (4–1,494) IU/L; platelet counts, 182 (40–483) × 10^3/mm^3; and HBV markers were HBsAg, 3,400 (0.06–27,700) IU/mL; and HBV DNA, 6.2 (<2.1 to >9.1) log copies/mL. HBeAg was not detectable in 5.4 % of studied patients, and the distribution of genotypes A/B/C/others was 4.5:15.6:79.6:0.3 %.

The HBsAg seroclearance rate analyzed by the Kaplan–Meier method was 9 % in 5 years, 17 % in 10 years, 27 % in 15 years, 35 % in 20 years, 44 % in 25 years, and 54 % in 30 years. The annual rate of HBsAg seroclearance was 1.75 % during 20 years (Fig. 1).

In the 2,112 patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were cirrhosis [relative risk (RR) 2.40 ($p = 0.014$)]; HBeAg negative [RR 3.01 ($p = 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.13 ($p = 0.004$)]. In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: HBeAg negative [RR 1.81 ($p < 0.001$)]; and HBsAg ≤2,000 IU/mL [RR 2.60 ($p < 0.001$)] (Table 2).

Untreated patients and treated patients

Differences in the baseline characteristics between 1,130 untreated and 982 treated patients are shown in Table 3: age [31 years vs. 36 ($p < 0.001$)]; male gender [62.4 vs. 71.9 % ($p < 0.001$)]; AST [median 27 vs. 56 IU/L ($p < 0.001$)]; ALT [median 28 vs. 96 IU/L ($p < 0.001$)]; γ-GTP [median 20 vs. 45 IU/L ($p < 0.001$)]; total bilirubin

Table 1 Baseline characteristics 2,112 patients infected with HBV followed for longer than 15 years

Features at the baseline	Patients (n = 2,112)
Demographic data	
Age (years)	37 (1–81)
Men	1,431 (67.8 %)
Liver disease	
Chronic hepatitis	2,031 (96.2 %)
Cirrhosis	81 (3.8 %)
Laboratory data	
AST (IU/L)	43 (3–2,192)
ALT (IU/L)	62 (2–3,020)
γ-GTP (IU/L)	27 (4–1,494)
Total bilirubin (mg/dL)	0.7 (0.1–21.2)
Albumin (g/dL)	4.3 (1.1–5.8)
Platelets (×10^3/mm^3)	182 (40–483)
α-Fetoprotein (μg/L)	4 (1–2,060)
HBV markers	
HBeAg-negative status	1,169 (55.4 %)
HBsAg (IU/mL)	3,400 (0.06–277,000)
HBcrAg (log U/mL)	5.4 (<3.0 to >6.8)
Genotypes (A/B/C/others)	4.5 %/15.6 %/79.6 %/0.3 %
HBV DNA (log copies/mL)	6.2 (<2.1 to >9.1)

Median values with the range in parentheses or numbers with the percentage in parentheses are given

HBV hepatitis B virus, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

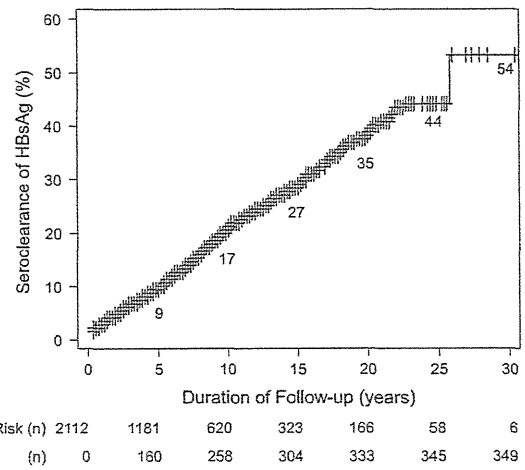

Fig. 1 Seroclearance of HBsAg in the 2,112 patients studied. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

Table 2 Factors influencing the seroclearance of HBsAg in 2,112 patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	*p* value
Age ≥50 years	1.06 (0.64–1.76)	0.824		
Male gender	1.15 (0.69–1.90)	0.594		
No HBV infection in family	1.55 (0.93–2.57)	0.092		
Treatment	1.26 (0.72–2.19)	0.413		
Cirrhosis	2.40 (1.20–4.83)	0.014		
AST ≥50 IU/L	1.30 (0.66–2.57)	0.454		
ALT ≥50 IU/L	1.81 (0.89–3.70)	0.104		
γ-GTP ≥20 IU/L	1.26 (0.72–2.23)	0.418		
Total bilirubin ≥1 mg/dL	1.39 (0.69–2.79)	0.358		
Albumin ≥4 g/dL	1.03 (0.58–1.81)	0.927		
Platelets >150 × 10^3/mm^3	1.22 (0.68–2.18)	0.501		
α-Fetoprotein ≤10 μg/L	1.06 (0.59–1.89)	0.845		
Genotype A or B, C	1.55 (0.86–2.76)	0.142		
HBeAg-negative status	3.01 (0.79–2.07)	0.001	1.81 (1.30–2.77)	<0.001
HBV DNA ≥5 log copies/mL	1.17 (0.64–2.15)	0.612		
HBsAg ≤2,000 IU/mL	2.13 (1.27–3.56)	0.004	2.60 (1.94–3.50)	<0.001
HBcrAg ≥4 log U/mL	1.11 (0.61–2.03)	0.731		
Wild-type precore sequence	0.98 (0.59–1.53)	0.964		
Wild-type core promoter sequence	2.74 (0.80–9.30)	0.104		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

[median 0.5 vs. 0.7 mg/dL ($p < 0.001$)]; albumin [median 4.4 vs. 4.3 g/dL ($p < 0.001$)]; platelets [median 202 vs. 181 × 10^3/mm^3 ($p < 0.001$)]; α-fetoprotein [median 4 vs. 4 μg/L ($p < 0.001$)]; HBeAg-negative status [75.8 vs. 31.8 % ($p < 0.001$)]; HBsAg levels [median 2,240 vs. 5,270 IU/mL ($p < 0.001$)]; HBcrAg [median 3.6 vs. >6.8 log U/mL ($p < 0.001$)]; distribution of genotypes A/B/C/others (5.7/20.0/72.6/1.7 vs. 3.4/11.1/84.9/0.5 %, $p < 0.001$); and HBV DNA [median 4.7 vs. 8.0 log copies/mL ($p < 0.001$)].

The rate of HBsAg seroclearance in treated patients was 8 % in 5 years, 20 % in 10 years, 28 % in 15 years, 41 % in 20 years, 49 % in 25 years, and 49 % in 30 years, with an annual HBsAg seroclearance rate of 2.05 % (Fig. 2). The rate in untreated patients was 9 % in 5 years, 18 % in 10 years, 26 % in 15 years, 33 % in 20 years, 42 % in 25 years, and 56 % in 30 years, with an annual HBsAg seroclearance rate of 1.65 %. No differences in the annual HBsAg seroclearance rate were noted between treated and untreated patients ($p = 0.289$).

HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, HBsAg persisted in 930 (82.3 %), whereas HBsAg seroclearance occurred in 200 (17.7 %). In the baseline characteristics, significant differences were found for age ($p < 0.001$), male gender ($p = 0.003$), chronic hepatitis ($p = 0.020$), γ-GTP ($p < 0.001$), albumin ($p = 0.004$), HBV genotypes ($p < 0.001$), HBeAg-negative status ($p < 0.001$), HBV DNA ($p < 0.001$), HBsAg level ($p < 0.001$), HBcrAg ($p < 0.001$), precore wild-type ($p < 0.001$), and core promoter wild-type ($p = 0.001$) (Table 4).

Factors contributing to HBsAg seroclearance in untreated patients

In the 1,130 untreated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.63 ($p = 0.002$)]; no family history in third-degree or closer relatives [RR 1.38 ($p = 0.037$)]; and HBsAg ≤2,000 IU/mL [RR 1.87 ($p < 0.006$)].

In multivariate analyses, only 2 factors contributed to HBsAg seroclearance: age ≥50 [RR 1.61 ($p = 0.018$)] and HBsAg ≤2,000 IU/mL [RR 1.77 ($p = 0.014$)] (Table 5).

HBsAg seroclearance in treated patients

In the 982 treated patients, HBsAg persisted in 833 (84.8 %). HBsAg seroclearance occurred in 149 (15.2 %). In the baseline characteristics, significant difference were found for male gender ($p = 0.004$), no family history in third-degree or closer relatives ($p = 0.010$), chronic hepatitis ($p = 0.001$), AST ($p = 0.010$), γ-GTP ($p = 0.023$), platelet counts ($p < 0.001$), HBeAg-negative status

Table 3 Baseline characteristics in untreated and treated patients

Features at the baseline	Untreated (n = 1,130)	Treated (n = 982)	Differences p value
Age (years)	31 (1–81)	36 (6–75)	<0.001
Men	705 (62.4 %)	726 (71.9 %)	<0.001
Chronic hepatitis	1,094 (96.8 %)	937 (96.4 %)	0.079
Cirrhosis	36 (3.2 %)	45 (3.6 %)	
AST (IU/L)	27 (3–1,776)	56 (6–2,192)	<0.001
ALT (IU/L)	28 (2–3,020)	96 (8–2,740)	<0.001
γ-GTP (IU/L)	20 (4–1,494)	45 (4–1,278)	<0.001
Total bilirubin (mg/dL)	0.5 (0.1–20.1)	0.7 (0.2–21.2)	<0.001
Albumin (g/dL)	4.4 (2.2–5.8)	4.3 (1.1–5.4)	<0.001
Platelets ($\times 10^3$/mm^3)	202 (40–443)	181 (40–483)	<0.001
α-Fetoprotein (μg/L)	4 (1–2,060)	4 (1–1,610)	<0.001
HBeAg-negative status	857 (75.8 %)	312 (31.8 %)	<0.001
HBsAg (IU/mL)	2,240 (0.06–141,000)	5,270 (0.09–277,000)	<0.001
HBcrAg (log U/mL)	3.6 (<3.0 to >6.8)	> 6.8 (<3.0 to >6.8)	<0.001
Genotypes [A/B/C/others (%)]	5.7/20.0/72.6/1.7	3.4/11.1/84.9/0.5	<0.001
HBV DNA (log copies/mL)	4.7 (<2.1 to >9.1)	8.0 (<2.1 to >9.1)	<0.001

Median values with the range in parentheses or numbers with the percentage in parentheses are given

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

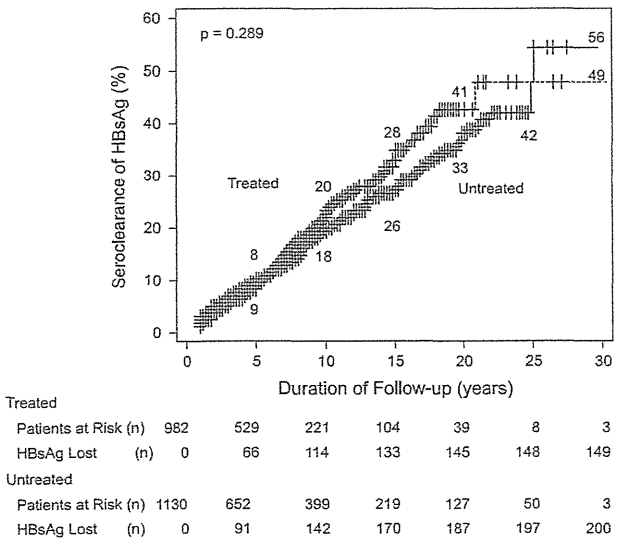

Fig. 2 Comparison of HBsAg seroclearance rates between 982 treated and 1,130 untreated patients. Numbers of patients at risk and those of patients who lost HBsAg are indicated below each time point

($p < 0.001$), HBV DNA ($p = 0.002$), HBsAg ($p < 0.001$), HBcrAg ($p = 0.003$), and precore wild-type ($p = 0.013$) (Table 6).

Factors contributing to HBsAg seroclearance in treated patients

In the 982 treated patients, factors influencing HBsAg seroclearance in univariate analysis by the Cox regression analyses were age ≥50 [RR 1.91 ($p = 0.001$)]; male gender [RR 2.14 ($p = 0.001$)], no family history in third-degree or closer relatives [RR 1.58 ($p = 0.005$)]; previous treatment with interferon [RR 2.13 ($p < 0.001$)]; chronic hepatitis [RR 3.12 ($p < 0.001$)]; AST ≥50 IU/L [RR 1.47 ($p = 0.031$)]; γ-GTP ≥20 IU/L [RR 1.87 ($p = 0.001$)]; platelets ≤150 × 10^3/mm^3 [RR 2.10 ($p < 0.001$)]; HBeAg-negative status [RR 2.53 ($p < 0.00$)]; HBV DNA ≤5 log copies/mL [RR 2.07 ($p = 0.001$)]; HBsAg ≤2,000 IU/mL [RR 2.29 ($p < 0.001$)]; HBcrAg ≤4 log U/mL [RR 2.28 ($p = 0.003$)]; and the wild-type precore sequence [RR 2.04 ($p = 0.011$)].

In multivariate analysis, only 3 factors contributed to HBsAg seroclearance: no family history in third-degree or closer relatives [RR 2.22 ($p = 0.006$)]; previous treatments with interferon [RR 3.15 ($p < 0.001$)]; and HBeAg-negative status [RR 3.75 ($p < 0.001$)] (Table 7).

Discussion

In Japan, perinatal materno-fetal transmission was the main route of HBV infection, but this transmission has been prevented since 1986 by the national campaign to prevent it by immunoprophylaxis with combined passive-active immunization of babies born to HBeAg-positive carrier mothers. However, HCC develops in about 10 % of the patients who have established chronic HBV infection by materno-fetal infection or through child-to-child transmission. Hence, HBsAg seroclearance is crucially required for preventing the development of cirrhosis followed by HCC.

In the present study, we analyzed 2,112 patients with persistent HBV infection to establish the factors

Table 4 Differences between the baseline characteristics of 917 untreated patients in whom HBsAg persisted and 213 those who lost HBsAg

Features at the baseline	HBsAg persisted ($n = 917$)	HBsAg lost ($n = 213$)	Differences p value
Age (years)	37 (1–81)	44 (0–80)	<0.001
Men	553 (60.3 %)	152 (71.4 %)	0.003
HBV in family members	349 (38.1 %)	76 (35.7 %)	0.509
Chronic hepatitis	893 (97.4 %)	201 (94.4 %)	0.020
AST (IU/L)	27 (3–1,144)	25 (6–1,776)	0.283
ALT (IU/L)	28 (6–1,960)	27 (6–3,020)	0.389
γ-GTP (IU/L)	22 (1–1,494)	29 (4–1,092)	<0.001
Total bilirubin (mg/dL)	0.6 (0.2–20.1)	0.7 (0.1–4.0)	0.257
Albumin (g/dL)	4.3 (2.0–5.3)	4.4 (1.6–5.7)	0.004
Platelets ($\times 10^3$/mm^3)	203 (40–443)	203 (33–417)	0.473
α-Fetoprotein (μg/L)	3 (1–2,060)	1 (1–478)	0.373
Genotypes [A/B/C/others (%)]	5.7/19.0/73.3/1.9	5.5/24.7/69.2/0.7	<0.001
HBeAg-negative status	663 (72.3 %)	194 (91.1 %)	<0.001
HBV DNA (log copies/mL)	4.9 (<2.1 to >9.1)	3.8 (<2.1 to >9.1)	<0.001
HBsAg (IU/mL)	3,100 (1.94–141,000)	149 (0.06–88,800)	<0.001
HBcrAg (log U/mL)	3.9 (<3.0 to >6.8)	2.9 (<3.0 to >6.8)	<0.001
Wild-type precore sequence	441 (48.1 %)	160 (75.0 %)	<0.001
Wild-type core promoter sequence	320 (34.9 %)	47 (22.0 %)	0.001

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 5 Factors influencing the seroclearance of HBsAg in untreated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.63 (1.19–2.23)	0.002	1.61 (1.09–2.37)	0.018
Male gender	1.08 (0.79–1.48)	0.618		
No HBV infection in family	1.38 (1.02–1.86)	0.037		
Cirrhosis	1.19 (0.73–1.93)	0.484		
AST ≥50 IU/L	1.01 (0.70–1.45)	0.979		
ALT ≥50 IU/L	0.93 (0.68–1.27)	0.633		
γ-GTP ≥20 IU/L	1.17 (0.85–1.61)	0.330		
Total bilirubin ≥1 mg/dL	1.41 (0.80–2.49)	0.239		
Albumin ≥4 g/dL	0.78 (0.51–1.18)	0.239		
Platelets >150 × 10^3/mm^3	0.99 (0.67–1.46)	0.946		
α-Fetoprotein ≤10 μg/L	0.84 (0.48–1.47)	0.543		
Genotype A or B	1.17 (0.81–1.69)	0.410		
HBeAg-negative status	0.78 (0.79–2.07)	0.314		
HBV DNA ≥5 log copies/mL	0.84 (0.58–1.24)	0.383		
HBsAg ≤2,000 IU/mL	1.87 (1.19–2.91)	0.006	1.77 (1.12–2.77)	0.014
HBcrAg ≥4 log U/mL	0.85 (0.50–1.45)	0.555		
Wild-type precore sequence	0.99 (0.60–1.52)	0.967		
Wild-type core promoter sequence	0.78 (0.35–1.73)	0.538		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

contributing to HBsAg seroclearance. The overall rate of HBsAg seroclearance was 1.75 % annually. The annual seroclearance rates of HBsAg are reported to be 1.7 % in Korea [14] and 1.6 % in Taiwan [15–17], as well as 2.5 % in Goto Islands of Japan, where HBV infections are very prevalent [18]. In 1,271 natives in Alaska, the rate of HBsAg seroclearance was 0.7 % annually [19]. These differences could be ascribed, in part, to HBV genotypes distinct among Asian countries and Alaska. Since treatment with IFN and/or nucleot(s)ide analogues has suppressive effects on the development of HCC [6, 20], they may influence HBsAg seroclearance.

Table 6 Differences in baseline characteristics between the 833 treated patients in whom HBsAg persisted and 149 those who lost HBsAg

Features at the baseline	HBsAg persisted (n = 833)	HBsAg lost (n = 149)	Differences p value
Age (years)	41 (13–88)	43 (17–71)	0.285
Men	601 (72.2 %)	124 (83.2 %)	0.004
HBV in family members	496 (59.6 %)	72 (48.3 %)	0.010
Chronic hepatitis	802 (96.3 %)	134 (89.9 %)	0.001
AST (IU/L)	54 (6–2,192)	78 (7–888)	0.010
ALT (IU/L)	93 (8–2,740)	118 (8–1,700)	0.117
γ-GTP (IU/L)	44 (4–1,278)	46 (4–1,278)	0.023
Total bilirubin (mg/dL)	0.7 (0.2–21.2)	0.7 (0.3–8.4)	0.273
Albumin (g/dL)	4.3 (1.1–5.4)	4.5 (1.4–5.3)	0.281
Platelets ($\times 10^3$/mm^3)	182 (40–483)	171 (50–391)	<0.001
α-Fetoprotein (μg/L)	4 (1–1,610)	4 (1–765)	0.682
Genotypes [A/B/C/others (%)]	3.2/10.7/85.1/1.0	5.1/12.4/81.6/0.9	0.565
HBeAg-negative status	230 (27.6 %)	79 (53.0 %)	<0.001
HBV DNA (log copies/mL)	7.8 (<2.1 to >9.1)	8.3 (<2.1 to >9.1)	0.002
HBsAg (IU/mL)	7,880 (0.04–277,000)	1,380 (0.04–188,000)	<0.001
HBcrAg (log U/mL)	6.9 (<3.0 to >6.8)	5.9 (<3.0 to >6.8)	0.003
Wild-type precore sequence	554 (66.6 %)	61 (41.2 %)	0.013
Wild-type core promoter sequence	274 (32.9 %)	67 (45.0 %)	0.836

Wild-type precore sequence, G1896; wild-type core promoter sequence, A1762/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Table 7 Factors influencing the seroclearance of HBsAg in treated patients evaluated by time-dependent uni- and multivariate analyses

Factors	Univariate analysis HBsAg clearance Relative risk (95 % CI)	p value	Multivariate analysis HBsAg clearance Relative risk (95 % CI)	p value
Age ≥50 years	1.91 (1.32–2.77)	0.001		
Male gender	2.14 (1.37–3.33)	0.001		
No HBV infection in family	1.58 (1.15–2.19)	0.005	2.22 (2.32–3.94)	0.006
Treatments (interferon vs. others)	2.13 (1.53–2.98)	<0.001	3.15 (1.69–5.87)	<0.001
Chronic hepatitis	3.12 (2.05–4.74)	<0.001		
AST ≥50 IU/L	1.47 (1.04–2.09)	0.031		
ALT ≥50 IU/L	1.29 (0.82–1.92)	0.201		
γ-GTP ≥20 IU/L	1.87 (1.30–2.70)	0.001		
Total bilirubin ≥1 mg/dL	1.35 (0.87–2.08)	0.179		
Albumin ≥4 g/dL	1.11 (0.66–1.86)	0.688		
Platelets ≤150 × 10^3/mm^3	2.10 (1.49–2.96)	<0.001		
α-Fetoprotein ≤10 μg/L	1.33 (0.92–1.92)	0.136		
Genotype A or B vs. others	1.16 (0.74–1.82)	0.529		
HBeAg-negative status	2.53 (1.83–3.50)	<0.001	3.75 (2.09–6.74)	<0.001
HBV DNA ≤5 log copies/mL	2.07 (1.37–3.13)	0.001		
HBsAg ≤2,000 IU/mL	2.29 (1.52–3.47)	<0.001		
HBcrAg ≤4 log U/mL	2.28 (1.31–3.97)	0.003		
Wild-type precore sequence	2.04 (1.18–3.55)	0.011		
Wild-type core promoter sequence	1.18 (0.63–2.21)	0.608		

Wild-type precore sequence, G1896; wild-type core promoter sequence, A176.2/G1764

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GTP* γ-guanosine triphosphate, *HBeAg* hepatitis B e antigen, *HBsAg* hepatitis B surface antigen, *HBcrAg* hepatitis B core-related antigen

Therefore, we went on to extend our analysis to untreated patients and those treated with IFN or nucleotide analogues separately. Criteria for upper or lower levels of each parameter were set, taking into consideration the median value or a cutoff value with the lowest p value of the entire 2,112-patient cohort (Table 1), and unified for untreated and treated patients (Tables 5, 7).

Firstly, in the univariate analysis, age, no family history of HBV infection in third-degree or closer relatives, and decreased HBsAg levels lowered the annual rate of HBsAg seroclearance significantly. In multivariate analysis, age $\geq$50 years (RR 1.61, $p = 0.018$) and HBsAg $\leq$2,000 IU/mL (RR 1.77, $p = 0.014$) decreased the annual rate of HBsAg seroclearance significantly. Kato et al. [18] reported high HBsAg seroclearance rates in patients over 40 or over 50 years; in our patients, also, age $\geq$50 years increased RR to 1.61 ($p = 0.018$). As for HBsAg and HBV DNA, low HBsAg and HBV DNA levels increased the HBsAg seroclearance rate to 37.7 %, and therefore, low HBsAg levels are an important factor. In actuality, HBsAg levels $\leq$2,000 IU/mL increased the rate of HBsAg seroclearance with RR 1.77 ($p = 0.014$).

In treated patients, by contrast, age, the male gender, no HBV infections in third-degree or closer relatives, treatment with IFN, chronic hepatitis, high AST levels, high γ-GTP levels, low platelet counts, HBeAg-negative status, low HBsAg levels, low HBcrAg levels and the wild-type precore sequence were significant factors in univariate analysis. In multivariate analysis, no HBV infections in third-degree or closer relatives (RR 2.22, $p = 0.006$), interferon treatments (RR 3.15, $p < 0.001$), and HBeAg-negative status (RR 3.75, $p < 0.001$) were significant factors.

Thus, there were differences in factors predictive of the HBsAg loss between untreated and treated patients. Remarkably, age and HBsAg titer were independent factors in untreated patients, whereas family history and negative HBeAg were independent factors in treated patients. Since this work studied patients who were followed for a long time (>15 years), age and HBsAg titer were factors for clearance of HBsAg in untreated patients. Treated patients, in contrast, would have included more patients with HBeAg, with a good response to antiviral treatment, as well as those without family history who would have been infected with HBV with a sorter duration than those with family history. In other words, most untreated patients were those with favorable clinical course, in whom HBsAg titer gradually decreased and eventually lost it with time. In fact, there would be many such patients, the majority of whom do not visit hospitals and are unaware of HBV infection, who may have unapparent liver disease. Treated patients, on the other hand, would have had higher risks for cirrhosis and HCC, owing to elevated ALT/AST levels; this risk is especially high for patients with a family history of HBV [21]. Therefore, patients with family history would not be able to easily lose HBsAg.

In treated patients, IFN led to HBsAg loss more effectively than other treatments [RR 2.13, $p < 0.001$ (Table 7)]. The immunomodulatory activity of IFN, which is not shared by nucleot(s)ide analogues, would have accelerated the immune response to HBV required for the seroclearance of HBsAg. Of the 333 patients who received IFN, 190 (57 %) were treated with IFN multiply. In them, seroclearance of HBsAg was achieved in 49 of the 190 (26 %) patients with multiple IFN treatments in comparison with 41 of the 143 (29 %) with single IFN treatment. Owing to indications for IFN, patients who received IFN tended to be younger, without previous treatments and higher HBV DNA as well as ALT levels. They might have increased the rate of HBsAg loss that was higher with IFN than other treatments.

Since this is a retrospective cohort study of patients visiting our hospital for more than 15 years, and there has been so much innovation in the treatment of chronic hepatitis B during that period, treated and untreated patients have different backgrounds at the baseline. Hence, treated patients had higher ALT and HBV DNA levels with severer liver disease than untreated patients (Table 3). This might have been responsible, at least in part, for the failure in finding differences in the rate of HBsAg loss between untreated and treated patients (Fig. 2). Future studies will be aimed at analyzing contributing factors in treated and matched controls. This will allow us to analyze factors contributing to HBsAg seroclearance in the treatment of patients with chronic hepatitis B.

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Conflict of interest These authors disclose the following: Dr. Kumada reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co., MSD KK, Bristol-Myers Squibb, Pharma International, Dentsu Sudler, and Hennessey Inc. Dr. Ikeda reports having received investigator, lecture, and consulting fees from Dainippon Sumitomo Pharma Co. No other potential conflicts of interest relevant to this article were reported.

References

1. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. N Engl J Med. 2004;350: 1118–29.
2. Lee WM. Hepatitis B virus infection. N Engl J Med. 1997;337: 1733–45.
3. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. 2006;295:65–73.

4. Huo TI, Wu JC, Lee PC, Chau GY, Lui WY, Tsay SH, et al. Seroclearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. Hepatology. 1998;28:231–6.
5. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology. 2006;130:678–86.
6. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, et al. Long-term outcome after hepatitis B surface antigen seroclearance in patients with chronic hepatitis B. Am J Med. 2006;119:71.e9–16.
7. Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. Gastroenterology. 2002;123:1084–9.
8. Chu CM, Liaw YF. HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. Hepatology. 2007;45:1187–92.
9. Yuen MF, Wong DK, Fung J, Ip P, But D, Hung I, et al. HBsAg seroclearance in chronic hepatitis B in Asian patients: replicative level and risk of hepatocellular carcinoma. Gastroenterology. 2008;135:1192–9.
10. Suzuki F, Arase Y, Suzuki Y, Akuta N, Sezaki H, Seko Y, et al. Long-term efficacy of interferon therapy in patients with chronic hepatitis B virus infection in Japan. J Gastroenterol. 2012;47:814–22.
11. Kobayashi M, Suzuki F, Akuta N, Hosaka T, Sezaki H, Yatsuji H, et al. Loss of hepatitis B surface antigen from the serum of patients with chronic hepatitis treated with lamivudine. J Med Virol. 2007;79:1472–7.
12. Akuta N, Suzuki F, Suzuki Y, Sezaki H, Hosaka T, Someya T, et al. Favorable efficacy of long-term lamivudine therapy in patients with chronic hepatitis B: an 8-year follow-up study. J Med Virol. 2005;75:491–8.
13. Hosaka T, Suzuki F, Kobayashi M, Seko Y, Kawamura Y, Sezaki H, et al. Clearance of hepatitis B surface antigen during long-term nucleot(s)ide analog treatment in chronic hepatitis B: results from a nine-year longitudinal study. J Gastroenterol. 2012. doi:10.1007/s00535-012-0688-7.
14. Ahn SH, Park YN, Park JY, Chang HY, Lee JM, Shin JE, et al. Long-term clinical and histological outcomes in patients with spontaneous hepatitis B surface antigen seroclearance. J Hepatol. 2005;42:188–94.
15. Kim JH, Lee JH, Park SJ, Bae MH, Kim JH, Kim do Y, et al. Factors associated with natural seroclearance of hepatitis B surface antigen and prognosis after seroclearance: a prospective follow-up study. Hepatogastroenterology. 2008;55:578–81.
16. Liaw YF, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. Hepatol Int. 2008;2:263–83.
17. Liu J, Yang HI, Lee MH, Lu SN, Jen CL, Wang LY, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance: a community-based follow-up study. Gastroenterology. 2010;139:474–82.
18. Kato Y, Nakao K, Hamasaki K, Kato H, Nakata K, Kusumoto Y, et al. Spontaneous loss of hepatitis B surface antigen in chronic carriers, based on a long-term follow-up study in Goto Islands, Japan. J Gastroenterol. 2000;35:201–5.
19. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska natives chronically infected with hepatitis B virus. Ann Intern Med. 2001;135:759–68.
20. Simonetti J, Bulkow L, McMahon BJ, Homan C, Snowball M, Negus S, et al. Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. Hepatology. 2010;51:1531–7.
21. Shiraki K, Yoshihara N, Sakurai M, Eto T, Kawana T. Acute hepatitis B in infants born to carrier mothers with the antibody to hepatitis B e antigen. J Pediatr. 1980;97:768–70.

CASE REPORT

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Transcatheter Arterial Chemotherapy with Miriplatin for Hepatocellular Carcinoma Patients with Chronic Renal Failure: Report of Three Cases

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Miriplatin is a novel lipophilic platinum complex that was developed to treat hepatocellular carcinoma (HCC). Although HCC patients frequently have coexisting chronic renal failure, little prospective data are available regarding the clinical toxicity of chemotherapeutic agents used to treat HCC patients with chronic renal failure. In a phase II study, the plasma concentration of total platinum in patients who received miriplatin was very low, and no severe renal toxicity caused by miriplatin injection was reported. Here, we present three cases of HCC with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin. All cases were male, ages 72, 84, and 83 years, and had serum creatinine levels of 2.3, 1.6, and 1.9 mg/dL, respectively. Their estimated glomerular filtration rates were 21.9, 20.3, and 22.2 mL/min, respectively. All cases were treated for unresectable HCC with transcatheter arterial chemotherapy with miriplatin. No serious adverse events were observed, and serum creatinine levels did not elevate, even in the patient who experienced renal failure caused by cisplatin administration. These results might suggest that transcatheter arterial chemotherapy with miriplatin can be safely used in HCC patients with chronic renal failure. **(Gut Liver 2013;7:246-251)**

Key Words: Miriplatin; Chronic renal failure; Hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide.[1] Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation [RFA]) are applicable in only 30% to 40% of HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC.[2-7] HCC patients frequently have coexisting cirrhosis, which is a predisposing factor for the development of renal dysfunction due to intravascular volume depletion, inadequate renal vasoconstriction, and hyperaldosteronism.[8-13]

Little prospective data are available regarding the clinical toxicity of chemotherapeutic agents used to treat HCC patients with chronic renal failure. Although cisplatin is an effective anticancer drug that is widely used for the treatment of many malignancies, including HCC, it is associated with significant nephrotoxicity, particularly in patients with chronic renal failure.[2,7] Miriplatin is a novel cisplatin derivative containing platinum with a high affinity for the iodized ethyl ester of fatty acids of poppyseed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) that is used in TACE. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC.[14-19]

In a Phase II HCC study, the plasma concentration of total platinum in patients receiving miriplatin was very low, and no severe renal toxicity caused by miriplatin injection was reported.[17] Here we present three cases of HCC with stage 4 chronic renal failure who received transcatheter arterial chemotherapy with miriplatin.[20]

CASE REPORTS

1. Case 1

A 72-year-old man with HCC, liver cirrhosis, and diabetic nephropathy had undergone RFA four times and TACE three times over 5 years. As shown in Fig. 1, a computed tomography (CT) scan of the liver revealed multiple HCCs (tumor size, 15 to 34 mm; tumor number, three; stage, T2N0M0). The serum creati-

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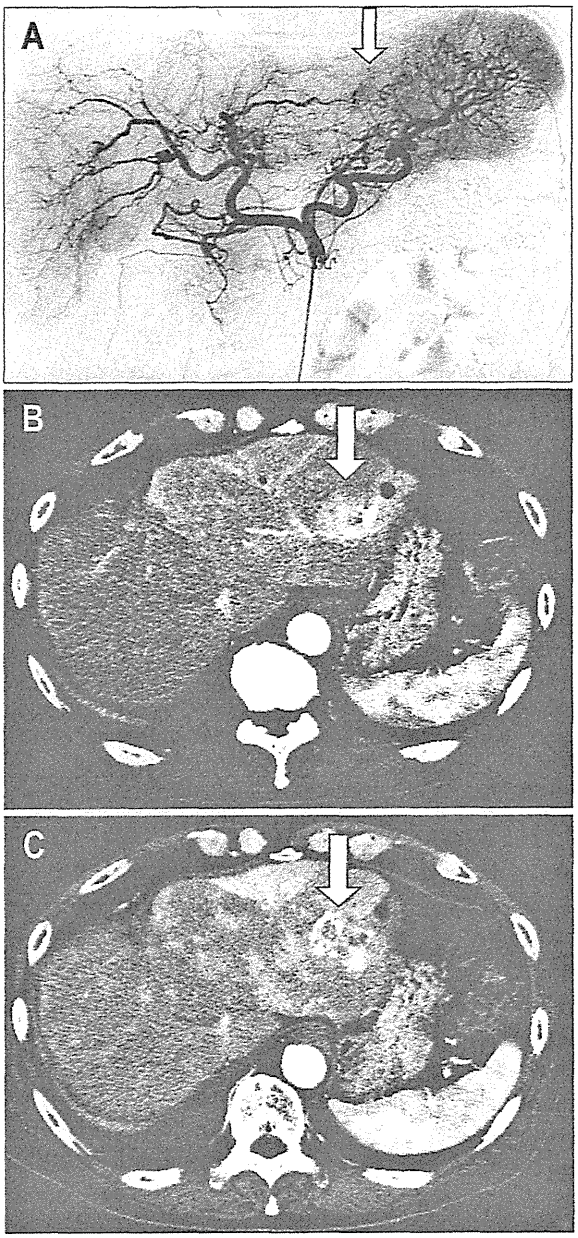

Fig. 1. Case 1. A 72-year-old man with unresectable hepatocellular carcinoma (HCC) who received transcatheter arterial chemoembolization (TACE) with miriplatin. (A) Abdominal angiography showed multiple HCCs (arrow). (B) Computed tomography (CT) showed multiple HCCs (arrow). (C) CT performed 1 month after TACE. The lesions revealed accumulations of lipiodol (arrow). Treatment efficacy was assessed as a partial response.

nine level was 2.3 mg/dL, and the estimated glomerular filtration rate (GFR) was 21.9 mL/min (Table 1).[21]

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension and 1 mm gelatin particles (1 mm-Gelpart; Nippon Kayaku, Tokyo, Japan). Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction. The patient received TACE with miriplatin (miriplatin 50 mg, lipiodol 2.5 mL, and 1 mm-Gelpart were injected from both the right and left hepatic arteries). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). Major side effects included grade 1 fever, elevated blood glucose, and grade 1 nausea, which all resolved within 1 week (the National Cancer Institute's Common Terminology Criteria for Adverse Events [CTCAE] version 4.0). Treatment efficacy was assessed 1 month after treatment. Partial response (modified response evaluation criteria in solid tumors, mRECIST) was achieved in all target lesions.[22]

The patient was received two times TACE with miriplatin at intervals of 4 months after the first administration (second and third dosage of miriplatin were 120 mg and dosage of lipiodol were 6 mL). The patient's weight and serum creatinine level still remained stable after repeat injection of miriplatin (serum creatinine level was 2.2 mg/dL after third TACE with miriplatin). Stable disease (mRECIST) was achieved in all target lesions after third TACE with miriplatin.

2. Case 2

An 84-year-old man with HCC, liver cirrhosis, and chronic renal failure had undergone RFA three times and TACE six times over 10 years. As shown in Fig. 3, a CT scan of the liver showed multiple HCCs (tumor size, 12 to 55 mm; tumor number, six; stage, T3N0M0). The serum creatinine level was 1.6 mg/dL, and the estimated GFR was 20.3 mL/min (Table 1).

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension. Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

The patient received transcatheter arterial chemotherapy with miriplatin (miriplatin 50 mg and lipiodol 2.5 mL were injected from both the right and left hepatic arteries). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). The major side effect of treatment was grade 1 fever, which resolved within 1 week (CTCAE version 4.0). Treatment efficacy was assessed 2 months after therapy. Stable disease (mRECIST) was achieved in all target lesions.

3. Case 3

An 83-year-old man with HCC, liver cirrhosis, hypertension,

Table 1. Patient Characteristics

Characteristic	Case 1	Case 2	Case 3
Age	72	84	83
Gender	Male	Male	Male
Height, cm	159	160	162
Weight, kg	58	47	57
Serum creatinine, mg/dL*	2.3	1.6	1.9
Estimated GFR1, mL/min†	21.9	20.3	22.2
Estimated GFR2, mL/min‡	22.8	32.5	27.0
Etiology	HCV	HCV	HBV
Child-Pugh score	A (6)	A (5)	A (5)
ICG-R15, %	16	13	4
Underlying disease that caused renal failure	Diabetic nephropathy	Chronic glomerulonephritis	Cisplatin induced renal failure
Tumor no.	3	6	40
Maximum tumor size, mm	34	55	39
Cancer stage (TNM)	II (T2N0M0)	III (T3N0M0)	II (T2N0M0)
Dosage of miriplatin, mg	100	100	70
Dosage of lipiodol, mL	5	5	3.5
Use of gelatin sponge particles	Yes	No	Yes
Contrast medium, mL	Iomeprol 60	Iomeprol 50	Iomeprol 190
Use of hydration therapy after miriplatin infusion	Yes	Yes	Yes

GFR, glomerular filtration rate; HCV, hepatitis C virus; HBV, hepatitis B virus; ICG-R15, indocyanine green retention rate at 15 minutes.
*Enzymatic method; †Cockcroft and Gault formula; ‡Japanese equation for estimating GFR.

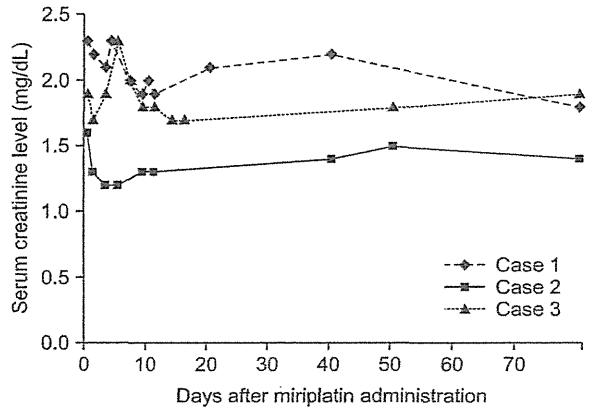

Fig. 2. Serum creatinine level after miriplatin administration in the three cases.

and renal failure that had been caused by cisplatin administration had undergone TACE nine times over 4 years. As shown in Fig. 4, a magnetic resonance imaging scan of the liver revealed multiple HCCs (tumor size, 5 to 39 mm; tumor number, 40; stage, T2N0M0). The patient's serum creatinine level was 1.9 mg/dL, and the estimated GFR was 22.2 mL/min (Table 1).

The patient was hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the miriplatin/lipiodol suspension and 1 mm-Gelpart. Miriplatin/lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

The patient received TACE with miriplatin (miriplatin 30 mg, lipiodol 1.5 mL, and 1 mm-Gelpart were injected from the right and left hepatic arteries, and miriplatin 10 mg and lipiodol 0.5 mL were injected from the right inferior phrenic artery). Therapy was well tolerated, and the patient's weight and serum creatinine level remained stable after treatment (Fig. 2). Major side effects included grade 1 fever and grade 1 nausea, both of which resolved within 1 week (CTCAE version 4.0). Treatment efficacy was assessed 3 months after therapy. Stable disease (mRECIST) was achieved in all target lesions.

DISCUSSION

Various anticancer drugs, such as doxorubicin hydrochloride, epirubicin hydrochloride, mytomycin C, cisplatin, and neocarzinostatin, have been used at TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified.

Miriplatin is a novel lipophilic cisplatin derivative that can be suspended in lipiodol and used for transcatheter arterial che-