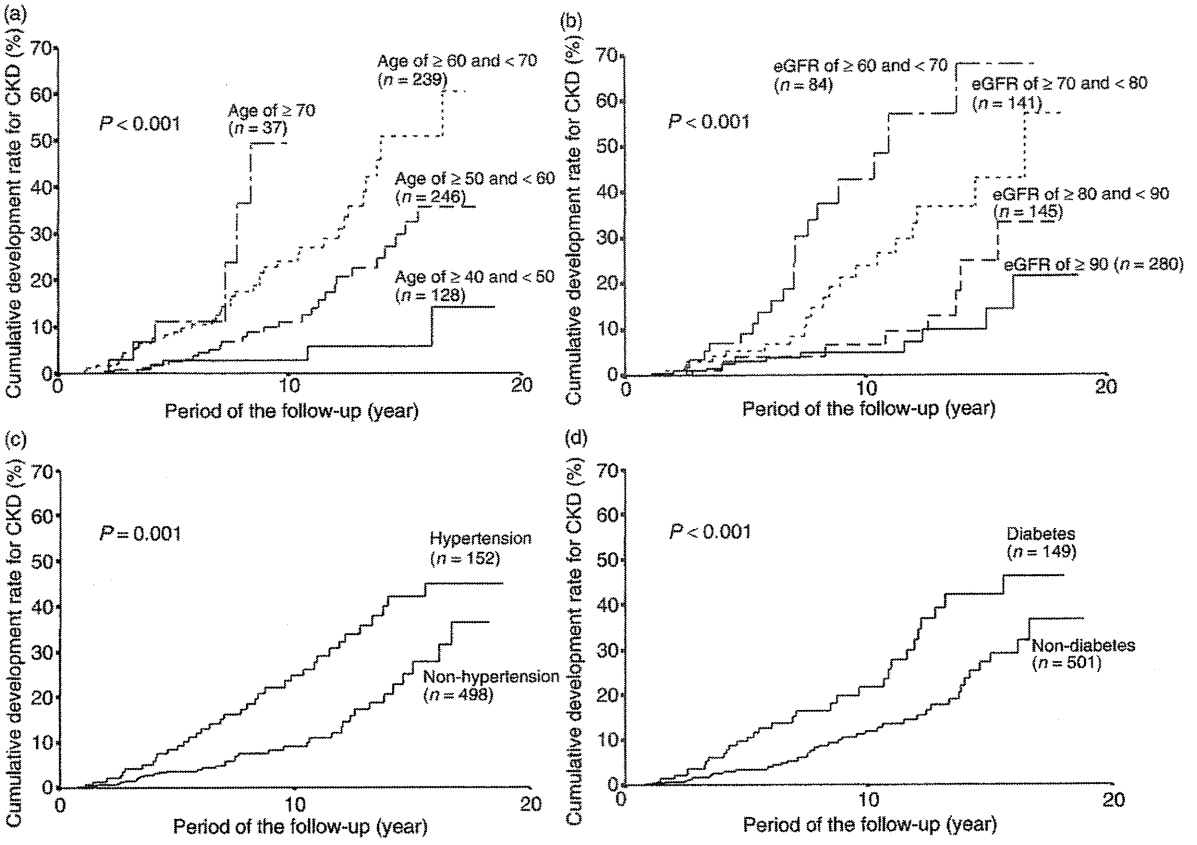

Figure 3 Cumulative development rate for chronic kidney disease (CKD) in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon: (a) Cumulative development rate for CKD based on difference of age; (b) Cumulative development rate for CKD based on the difference of estimated glomerular filtration rate (eGFR); (c) Cumulative development rate for CKD based on the difference of blood pressure; (d) Cumulative development rate for CKD based on the difference of glucose level.

health check programs in 2000–2004, from seven different prefectures in Japan. Next, the prevalence of CKD stage 3 in the study population, stratified by age groups of 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and 80–89 years, were 1.4%, 3.6%, 10.8%, 15.9%, 31.8%, 44.0%, and 59.1%, respectively. Moreover, they provided that the prevalence of stage 4+5 was <0.2%. Our results agreed with Imai's report in the fact that end-stage CKD patients were few.

The present study was limited by a retrospective cohort trial. This cohort is over 10 years; hence, many patients had complications, such as diabetes and hypertension. However, the development of CKD was mainly evaluated based on the clinical characteristics at the initiation of follow-up. Second limitation of the study was that we defined CKD according to eGFR alone. Gener-

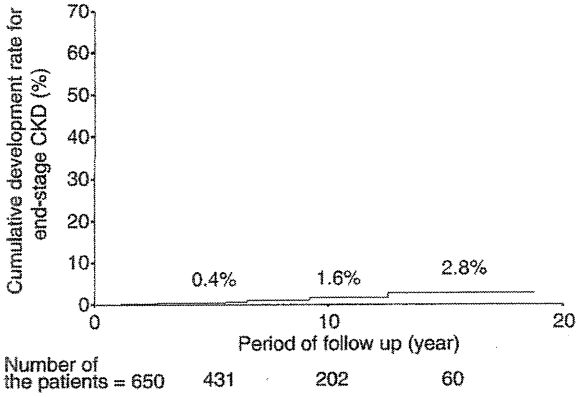

Figure 4 Cumulative development rate for end-stage chronic kidney disease (CKD) based on the difference of efficacy in hepatitis C virus (HCV) positive and cirrhotic patients treated with interferon.

Table 3 Predictive factors for end-stage chronic kidney disease (CKD) development

Variables	Univariate analysis	
	HR (95%CI)	*P*
Age, per 10 years	2.13 (0.86–5.30)	0.104
Sex (female/male)	0.24 (0.03–1.92)	0.182
Body mass index (≥25/<25)	0.80 (0.16–4.10)	0.782
HCV load (KIU/mL, ≥1000/<1000)	1.58 (0.37–6.67)	0.535
Genotype (1/2)	2.74 (0.66–11.50)	0.167
AST (IU/L, ≥50/<50)	1.45 (0.18–11.76)	0.730
ALT (IU/L, ≥50/<50)	1.89 (0.45–7.93)	0.382
Platelet ($\times 10^4/mm^3$, ≥15/<15)	0.67 (0.16–2.86)	0.586
eGFR, per decrease of 10 mL/min/1.73 m^2	1.70 (0.89–3.23)	0.105
Uric acid (mg/dL, ≥7.0/<7.0)	1.27 (0.23–6.96)	0.784
Triglyceride (mg/dL, ≥150/<150)	1.33 (0.15–11.87)	0.802
Cholesterol (mg/dL, ≥220/<220)	1.03 (0.12–8.67)	0.980
Diabetes (+/–)	1.89 (0.45–7.93)	0.382
Hypertension (+/–)	2.83 (0.70–11.41)	0.143
Combination of ribavirin (+/–)	0.88 (0.10–7.66)	0.908
Kind of IFN (beta/alpha)	2.08 (0.52–8.37)	0.300
Efficacy (non-SVR/SVR)	3.25 (0.40–26.4)	0.269
Frequencies of contrast imaging per year (≥1/<1)	3.72 (0.70–19.72)	0.123

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; HR, hazards ratio; IFN, interferon; SVR, sustained virological response.

ally, a recent definition of CKD also includes proteinuria.[28,29] Although the use of both eGFR and proteinuria might lead to a more accurate classification of CKD, we could not assess proteinuria in this study. Third, prescribed agents during the follow-up were not considered in the present study. However, therapy intervention is very important for protecting new development for CKD. In future, the intervention therapy for protecting the development of CKD should be evaluated. Finally, in the present study, patients were treated with different types of antivirus therapy (IFN monotherapy or combination therapy of IFN and ribavirin) for different durations (4 weeks to 52 weeks). This heterogeneity makes it slightly difficult to interpret the results of the study. On the other hand, the strengths of the present study are a long-term follow-up in the large numbers of patients included.

The present study shows several findings with regard to development incidence for CKD or end-stage CKD after the termination of IFN therapy for HCV positive and cirrhotic patients. First, SVR is effective for protecting the development incidence for CKD in HCV patients with liver cirrhosis. Though the role of HCV in the pathogenesis of aggravation of renal function remains speculative, the following possible mechanism have been reported: (i) systemic immune response to HCV infection mediated by cryoglobulins, HCV-antibody immune complexes, or amyloid deposition;[8,30,31] (ii) toll-like receptors increased expression in glomeruli induce immune response;[32] and (iii) insulin resistance and hyperinsulinemia cause excess intrarenal production of insulin-like growth factor-1 and transforming growth factor β, thus induce oxidative stress.[33] In addition, patients with liver cirrhosis might have the possibility of kidney damage such as hypovolemia due to fluid loss or hemorrhage, hepatorenal syndrome, and drug-induced renal failure. Second, in addition to non-SVR, the present study suggests that aging, low eGFR, hypertension, and diabetes enhanced the development of worsening renal function in cirrhotic patients with HCV infection after the termination of IFN. The repeated use of contrast imaging of computed tomography might worsen renal function. However, in the present study, SVR, aging, low eGFR, hypertension, and diabetes were the main predictive factors for the development of CKD compared to the repeated use of contrast imaging of computed tomography. The result that aging, hypertension and diabetes were associated with the development of worsening renal function agreed with several studies.[16–19]

In the present study, the predictive factors for end-stage CKD (stage 5) were not similar to those for CKD 3–5. The possible reason for this discrepancy is as follows. First, the number of patients who had progressed to end-stage CKD was six. Because of so few patients, we could not show the statistical significance in the predictive factors for end-stage CKD. Second, development of end-stage CKD might be robust to the several factors at the initiation of the follow-up. Development of end-stage CKD might be associated with the accidents during follow-up, such as the repeated use of contrast medium and hypovolemia due to bleeding. In fact, four of six patients who progressed to end-stage CKD had been given the repeated use of contrast medium. Next, whether HCV eradication in patients whose renal function progressed to stage of CKD 3–5 improves the mortality due to cardiovascular disease and stroke is a very important issue. However, this problem was not evaluated in the present study. This should be clarified by further examination.

In conclusion, our study suggests that the annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of CKD.

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Previous Chemoembolization Response after Transcatheter Arterial Chemoembolization (TACE) Can Predict the Anti-Tumor Effect of Subsequent TACE with Miriplatin in Patients with Recurrent Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma · Miriplatin · Transcatheter arterial chemoembolization

Abstract
Aim: The purpose of this retrospective study was to evaluate the efficacy and safety of transcatheter arterial chemoembolization (TACE) with miriplatin in patients with unresectable hepatocellular carcinoma (HCC). ***Methods:*** From 2007 to 2010, 122 consecutive patients with unresectable HCC were treated by TACE with miriplatin-lipiodol suspension in our institute. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. Ninety-eight patients (80%) had a history of TACE. ***Results:*** Thirty-five of the 122 treated patients (29%) showed complete response (CR). And no serious complications were observed. Patients who had shown CR after previous TACE (pre-CR) were significantly more likely to show CR in the current study compared with patients who had shown less successful responses after previous TACE (56 vs. 20%, p = 0.003). Multivariate analysis revealed that response after previous TACE (pre-CR, risk ratio: 4.76; p = 0.035), tumor multiplicity (solitary, risk ratio: 9.69; p = 0.003), and injection artery (peripheral to segmental hepatic artery, risk ratio: 5.28; p = 0.040) were significant independent predictors associated with CR after TACE using miriplatin. ***Conclusion:*** In repetition of TACE treatment, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown a CR after previous TACE.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide [1]. Since it is well known that more than 80% of HCC cases are associated with liver cirrhosis, a routine check-up including ultrasound for cirrhotic patients could potentially lead to the detection of early HCC [2–4]. Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation) are applicable in only 30–40% of

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HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC [5–12].

Although many chemotherapeutic agents (e.g. doxorubicin, epirubicin, mitomycin C, and cisplatin) are used with the ethyl ester of iodized fatty acids from poppy-seed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) in TACE, the best choices for first- and second-line drugs remain uncertain [13–15]. Miriplatin (cis-[((1R,2R)-1,2-cyclohexanediamine-N,N′)bis(myristato)]-platinum(II)monohydrate; Dainippon Sumitomo Pharma Co., Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent [16–19]. When lipiodol is injected into an artery feeding HCC nodules, it selectively accumulates in the tumor. Accordingly, a miriplatin-lipiodol emulsion is deposited within the HCC nodules and gradually releases active platinum compounds into tumor tissues. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC, but the efficacy of TACE using miriplatin for patients with recurrent HCC after TACE has not been evaluated [20, 21]. The purpose of this retrospective study was to evaluate the efficacy and safety of TACE using miriplatin for patients with HCC.

Patients and Methods

Study Population

From December 2007 to December 2010, 122 consecutive patients with unresectable HCC were treated by TACE with a miriplatin-lipiodol suspension at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. The study group consisted of 79 men and 43 women ranging in age from 48 to 87 years (median, 72 years). They included 11 patients (9%) positive for HBs-Ag, 103 patients (84%) positive for HCV antibody, and 8 patients (7%) negative for both. At the time of the miriplatin administration, median values were as follows: total bilirubin level = 1.1 mg/dl; serum albumin concentration = 3.3 g/dl; indocyanin-green retention rate at 15 min = 29%; prothrombin activity = 82.5%; alpha-fetoprotein (AFP) concentration = 31.2 ng/ml; and des-gamma-carboxyprothrombin (DCP) concentration = 53 AU/l. As for Child-Pugh classification, 92 patients (75%) were Class A and 30 patients (25%) were Class B. The clinical characteristics of the study group are summarized in table 1. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

Hepatocellular Carcinoma

Before treatment with miriplatin, all patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver-imaging studies (dynamic computerized tomography [dynamic CT], ultrasonography [US], digital-subtraction angiography [DSA]), complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of dynamic CT, US and DSA. Patients who had extrahepatic metastasis of HCC or other malignancies were excluded.

Tumor profiles and TACE treatment history for the study group are summarized in table 2. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. The median diameter of the largest tumor was 20 mm (range 10–100 mm). Ninety-eight patients (80%) had a history of TACE. Thirty-seven patients had received cisplatin, and 80 patients had received epirubicin. Among these patients, the median number of

Table 1. Demographic characteristics and pretreatment assessments of 122 patients who underwent TACE using a miriplatin/lipiodol suspension for unresectable HCC

Number of cases	122
Age, years	72 (48–87)
Gender, male	65%
Etiology, HCV/HBV/others	103/11/8
Child-Pugh Class, A/B/C	92/30/0
ICG-R15, %	29 (4–78)
Albumin, g/dl	3.3 (2.0–4.2)
Total bilirubin, mg/dl	1.1 (0.4–4.9)
Prothrombin activity, %	82.5 (45.7–123.1)
Platelet, $\times 10^3/\mu l$	93 (29–282)
AFP, ng/ml	31.2 (1.8–152,800)
DCP, AU/l	53 (6–65,290)

HCV = Hepatitis C virus; HBV = hepatitis B virus; ICG-R15 = indocyanine-green retention rate at 15 min.

Variables are expressed as medians with ranges in parentheses.

Table 2. Tumor profiles, treatment history, and study drug dosages of 122 patients who underwent TACE using miriplatin for unresectable HCC

Tumor size, mm	20 (10–100)
Intrahepatic multiplicity, solitary	22 (18%)
Number of tumors	4 (1–100)
Presence of portal vein invasion	3 (2%)
History of TACE	98 (80%)
History of TACE with epirubicin	80 (66%)
History of TACE with cisplatin	37 (30%)
Median interval between previous TACE and miriplatin administration, months	4 (1–41)
Dosage of miriplatin, mg	80 (20–120)
Dosage of lipiodol, ml	3 (1–6)
Injection from peripheral to segmental branch of the hepatic artery	22 (18%)

Variables are expressed as medians with ranges in parentheses or number of cases.

TACE procedures was four (range 1–13), and the median interval between previous TACE and miriplatin administration was 4 months (range 1–41 months).

Treatment Protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and the catheter was inserted superselectively into the hepatic artery that supplied the target tumor for injection of the miriplatin-lipiodol suspension and 1-mm gelatin cubes (Gelpart; Nippon Kayaku, Tokyo). The 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.

Assessment of Therapeutic Effects

The effect of chemotherapy was evaluated by dynamic CT 1 to 3 months after TACE with miriplatin, and was based on the change in the maximum diameter of the viable target lesions (i.e. showing enhancement in the arterial phase). Response categories, according to the criteria of Modified Response Evaluation Criteria in Solid Tumors (mRECIST) [22], are as follows: complete response (CR) = disappearance of any intratumoral arterial enhancement in all target lesions; partial response (PR) = at least a 30% decrease in the sum of diameters of viable target lesions; stable disease (SD) = any cases that do not qualify for either PR or progressive disease; and progressive disease (PD) = an increase of at least 20% in the sum of the diameters of viable target lesions.

Toxicity Evaluation

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria (version 4.0). Within 2 weeks before TACE with miriplatin, and at 3 to 7 days (three times during this period) and at 1 month afterward, the following toxicity evaluations were made: hematological assessments (i.e. leukocyte and thrombocyte counts) and clinical chemistry assessments (i.e. serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], albumin, total bilirubin, serum creatine, and prothrombin activity).

Statistical Analysis

The distribution of subject characteristics was assessed by the chi-square test or the Mann-Whitney's U test, as appropriate. Multivariate logistic regression analysis was used to evaluate significant factors for CR by TACE with miriplatin. All variables are expressed as mean (range). All tests were 2-sided, and p values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., IBM, Somers, N.Y., USA).

Results

Dosing of Study Drugs

Table 2 summarizes the profiles and study drug data of 122 HCC patients who were treated with miriplatin. The median dosage of miriplatin was 80 mg (range 20–120 mg), and the median dosage of lipiodol was 3 ml (range 1–6 ml). Twenty-two patients (18%) were injected with the miriplatin-lipiodol suspension from the peripheral to the segmental branch of the hepatic artery. Thirty patients (25%) were injected with the miriplatin-lipiodol suspension from the anterior or posterior segmental branch of the right hepatic artery. Sixty-six patients (54%) were injected with the miriplatin-lipiodol suspension from the right or left branch of the hepatic artery. And 4 patients (3%) were injected with the miriplatin-lipiodol suspension from the proper hepatic artery.

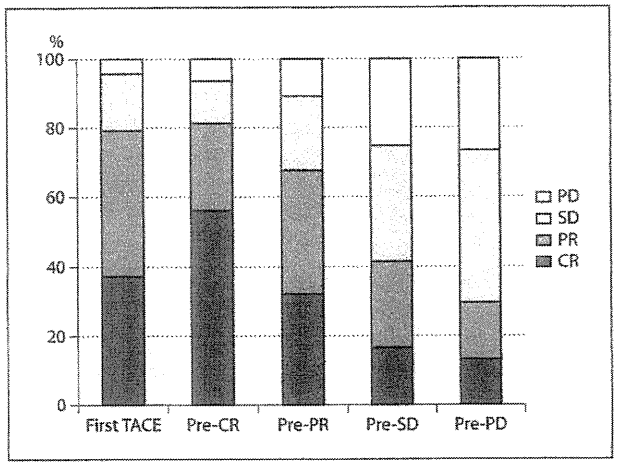

Fig. 1. The efficacy of TACE using miriplatin in patients with HCC according to response to previous TACE. Abbreviations used in the figure: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. pre-CR group (n = 16): patients who showed CR after previous TACE. pre-PR group (n = 28): patients who showed PR after previous TACE. pre-SD group (n = 24): patients who showed SD after previous TACE. pre-PD group (n = 30): patients who showed PD after previous TACE.

Treatment Effects

Thirty-five of the 122 treated patients (29%) showed CR, 35 patients (29%) showed PR, 33 patients (27%) showed SD, and 19 patients (15%) showed PD. Overall, 58% of patients showed an objective response (i.e. CR or PR).

Treatment Effects according to Previous TACE Effect

The efficacy of TACE using miriplatin according to the treatment effect of previous TACE was as follows (and is illustrated in fig. 1). For the first TACE group (patients who received TACE for the first time), 9 of 24 patients (38%) showed CR; for the pre-CR group (patients who

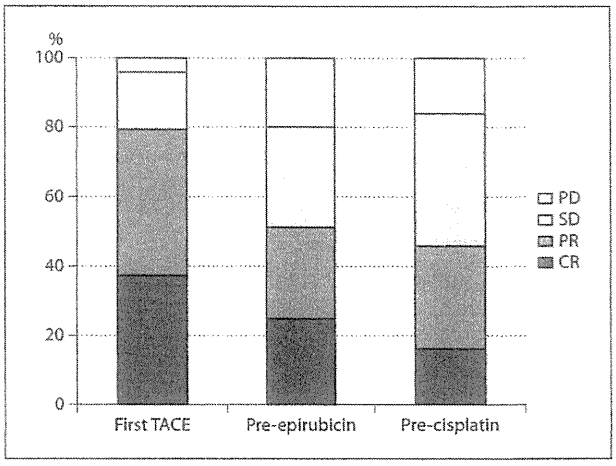

Fig. 2. The efficacy of TACE using miriplatin in patients with HCC according to previous TACE agent. Abbreviations used in the figure: CD = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. Pre-cisplatin group (n = 37): patients who had received TACE using cisplatin. Pre-epirubicin group (n = 80): patients who had received TACE using epirubicin.

showed CR response after previous TACE), 9 of 16 patients (56%) showed CR; for the pre-PR group (patients who showed PR response after previous TACE), 9 of 28 patients (32%) showed CR; for the pre-SD group (patients who showed SD response after previous TACE), 4 of 24 patients (17%) showed CR; and for the pre-PD group (patients who showed PD response after previous TACE), 4 of 30 patients (13%) showed CR.

Treatment Effects according to Previous TACE Agent

In patients who had received TACE using epirubicin, 20 of 80 patients (25%) showed CR and 21 of 80 patients (26%) showed PR. In patients who had received TACE using cisplatin, 6 of 37 patients (16%) showed CR and 11 of 37 patients (30%) showed PR. In each of the above groups, the objective response rate (sum of CR and PR) was significantly lower than that in patients who received their first TACE ($p = 0.015$ and $p = 0.010$, respectively), as illustrated in figure 2.

Univariate analysis identified the following six factors as influencing the rate of CR: response after previous TACE (pre-CR group vs. other groups, $p = 0.005$), tumor multiplicity (solitary vs. multiple, $p < 0.0001$), gamma-GTP concentration (≤40 vs. >40 IU/l, $p = 0.037$), AFP concentration (≤40 vs. >40 ng/ml, $p = 0.042$), DCP concentration (≤50 vs. >50 AU/l, $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, $p = 0.001$). These parameters were entered into multivariate logistic regression analysis, which revealed that response after previous TACE (pre-CR group vs. other groups, risk ratio: 4.76; 95% CI: 1.11–20.37; $p = 0.035$), tumor multiplicity (solitary vs. multiple, risk ratio: 9.69; 95% CI: 2.18–42.92; $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, risk ratio: 5.28; 95% CI: 1.07–25.95; $p = 0.040$) were significant independent predictors associated with CR after TACE using miriplatin (table 3).

Adverse Effects

Fever, anorexia, and elevation of serum transaminase levels were observed in most patients after miriplatin administration (table 4). The following Grade 4 events were observed: decreased neutrophil count in 1 patient (1%), increased AST in 4 patients (3%), and increased ALT in 1 patient (1%); all these cases resolved within 2 weeks. In this study group, no vascular complications of the hepatic artery were observed. No other serious complications or treatment-related deaths were observed after miriplatin administration.

Discussion

TACE is most widely performed in patients with HCC who are not eligible for curative therapy. The survival benefit of TACE has been confirmed by randomized controlled trials and meta-analyses. Various anti-cancer drugs, such as doxorubicin, epirubicin, mytomycin C, cisplatin, and neocarzinostatin, have been used as TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified [13–15].

Although TACE can be repeated in most patients, good therapeutic efficacy cannot be expected when the same anti-cancer drug is used more than once since various types of resistance to therapy can develop during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs [23, 24]. Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior anti-tumor effect in HCC with lower toxicity compared with cisplatin [16–

Table 3. Univariate and multivariate analysis of predictors of complete necrosis (logistic regression analysis)

Category	Univariate		Multivariate	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Tumor multiplicity, solitary vs. multiple	8.57 (3.08–23.8)	<0.0001	9.69 (2.19–42.9)	0.003
Response by pre-TACE, pre-CR vs. others	4.91 (1.59–15.1)	0.005	4.76 (1.11–20.3)	0.035
Injection artery, peripheral to segmental hepatic artery vs. others	2.50 (0.96–6.48)	0.001	5.28 (1.07–25.9)	0.040
DCP, ≤50 vs. >50 AU/l	4.04 (1.61–10.13)	0.003	3.55 (0.99–12.6)	0.051
gamma-GTP, ≤40 vs. >40 IU/l	2.39 (1.05–5.44)	0.037		
AFP, ≤40 vs. >40 ng/ml	2.50 (1.03–6.06)	0.042		

Table 4. Adverse effects after miriplatin administration

Grade:	1	2	3	4
White blood cell decreased	1 (1%)	27 (22%)	7 (6%)	0
Neutrophil count decreased	2 (2%)	21 (17%)	5 (4%)	1 (1%)
Anemia	40 (33%)	21 (17%)	3 (2%)	0
Platelet count decreased	72 (59%)	21 (17%)	11 (9%)	0
AST increased	55 (45%)	23 (19%)	30 (25%)	4 (3%)
ALT increased	54 (44%)	12 (10%)	19 (16%)	1 (1%)
Fever	67 (55%)	14 (11%)	0	0
Anorexia	56 (46%)	1 (1%)	0	0
Nausea	23 (19%)	0	0	0
Abdominal pain	22 (18%)	4 (3%)	0	0
Hepatic infection	0	0	1 (1%)	0

Values denote numbers of subjects. Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0.

19]. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miriplatin.

According to pharmacokinetic studies, the plasma concentration of total platinum is much lower in patients treated with miriplatin compared with that in patients treated with intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer for miriplatin than the corresponding values for intra-arterial cisplatin.

Miriplatin/lipiodol releases 1,2-diaminocyclohexane platinum (II) dichloride (DPC) as its active platinum compound, which binds to nuclear DNA and mediates miriplatin/lipiodol cytotoxicity. In a cisplatin-resistant rat hepatoma cell-line model, cross-resistance to DPC was not observed [25].

Prior to the current study, clinical trials have shown that miriplatin is effective for the treatment of HCC, but the efficacy of switching the TACE anti-cancer drug from epirubicin or cisplatin to miriplatin for a repeat TACE had not been evaluated.

In the present study, having a low number of tumors (solitary vs. multiple), receiving the treatment injection in the peripheral to segmental hepatic artery, and having shown complete tumor necrosis after prior TACE (pre-CR group) were highly correlated with complete tumor necrosis after TACE with miriplatin. A previous CR may be a surrogate marker for other factors, such as tumor sensitivity to anti-cancer agents and intra-hepatic metastasis. Among the 54 patients in this study who had shown no change or disease progression after previous TACE (pre-SD and pre-PD groups), 19 patients (35%) showed an

objective response by switching the TACE agent from epirubicin or cisplatin to miriplatin.

In repetition of TACE, vascular complications can cause development of parasitic feeding arteries for liver cancers leading to insufficient tumor embolization; rapid tumor growth may follow. In the present study, no vascular complications or other serious adverse events were observed. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Previous studies reported that complete tumor necrosis after TACE offered favorable long-term survival outcomes to HCC patients [7, 26]. In the current study, miriplatin administration was associated with a beneficial tumor response even in recurrent HCC after TACE. These results suggest that miriplatin administration may offer a favorable prognosis for recurrent HCC after TACE.

Conclusion

In repetition of TACE in HCC patients, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown CR after previous TACE. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Disclosure Statement

The following authors have received honoraria (lecture fee) from Dainippon Sumitomo Pharma Co., Ltd, Osaka, Japan: Hiromitsu Kumada, MD; Kenji Ikeda, MD; Yasuji Arase, MD; Yoshiyuki Suzuki, MD; Fumitaka Suzuki, MD; and Norio Akuta, MD.

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INTERNAL MEDICINE

ORIGINAL ARTICLE

The Development of Chronic Kidney Disease in Japanese Patients with Non-alcoholic Fatty Liver Disease

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Abstract

Objective Chronic kidney disease (CKD) is present in patients with nonalcoholic fatty liver disease (NAFLD). The aim of this retrospective study was to assess the cumulative development incidence and predictive factors for new onset of CKD in Japanese patients with NAFLD.

Methods A total of 5,561 NAFLD patients without CKD were enrolled. CKD was defined as either an estimated glomerular filtration rate of <60 mL/min/1.73 m^2 or dipstick proteinuria ($\geq +1$). A blood sample and a urine sample were taken for routine analyses during follow-up. The mean observation period was 5.5 years. The primary goal is the new development of CKD. Independent factors associated with new development of CKD were analyzed by using the Kaplan-Meyer method and the Cox proportional hazards model.

Results Of 5.561 NAFLD patients, 263 patients developed CKD. The cumulative development rate of CKD was 3.1% at the 5th year and 12.2% at the 10th year. Multivariate Cox proportional hazards analysis showed that CKD development in patients with NAFLD occurred when patient had low level of GFR of 60-75 mL/min/1.73 m^2 [hazard ratio:2.75; 95% confidence interval (CI) =1.93-3.94; p<0.001], age of ≥50 years (hazard ratio: 2.67; 95% CI=2.06-3.46; p<0.001), diabetes (hazard ratio: 1.92; 95% CI=1.45-2.54; p<0.001), hypertension (hazard ratio: 1.69; 95% CI=1.25-2.29; p<0.001), and elevated serum gamma-glutamyltransferase of ≥109 IU/L(hazard ratio: 1.35; 95% CI=1.02-1.78; p=0.038).

Conclusion Our retrospective study indicates that the annual incidence of CKD in Japanese patients with NAFLD is about 1.2%. Five factors of low eGFR level, aging, type 2 diabetes, hypertension, and elevated gamma-glutamyltransferase, increases the risk of the development of CKD.

Key words: nonalcoholic fatty liver disease, chronic kidney disease, gamma-glutamyltransferase

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the more common causes of chronic liver disease in Western world (1-4) and in many Asian nations (5, 6). NAFLD is considered to be the liver component of metabolic syndrome (7-9). It is associated with obesity, dyslipidemia, pituitary dysfunction, hypertension, sleep apnea, and type 2 diabetes mellitus (T2DM) (10-16). Moreover, NAFLD often causes cardiovascular disease and stroke (17, 18). Thus, NAFLD is emerging as a new significant health problem in many countries.

On the other hand, there has been a recent dramatic in-

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crease in the prevalence of end-stage renal disease (ESRD) in USA and Asia (19-22). Chronic kidney disease (CKD) often progresses to ESRD with its attendant complications. CKD, a disease entity including mild to ESRD due to any etiology, was defined as an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2 and/or the presence of proteinuria (21). Recently, metabolic syndrome and NAFLD have been reported to enhance the new onset of CKD (23, 24). Although there is growing evidence to support the concept that metabolic syndrome is a risk factor for developing CKD, little research has been done to evaluate whether NAFLD is associated with the long-term development of CKD.

The present cohort study was initiated to investigate the cumulative incidence and risk factors of CKD after long-term follow-up in patients with NAFLD. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Methods

Patients

The number of Japanese patients who were diagnosed with fatty liver by ultrasonography (25) between January 1997 and December 2007 in the Department of Hepatology and/or Health Management Center, Toranomon Hospital, Tokyo, Japan was 9,120. Of these, 5,561 Japanese patients satisfied with the following enrolled criteria; 1) no evidence of CKD based on eGFR calculated with serum creatinine level (eGFR≥60 [mL/min/1.73 m^2]); 2) the absence of proteinuria (≥ +1); 3) current and past daily alcohol intake of <20 g/day; 4) negativity for hepatitis B surface antigens (HBsAg), hepatitis C virus antibodies, antinuclear antibodies, or antimitochondrial antibodies in serum, as determined by radioimmunoassay, enzyme-linked immunosorbent assay or spot hybridization; 5) no underlying neoplasm or systemic disease, such as systemic lupus erythmatosus, rheumatic arthritis; 6) no evidence of nodules of hepatocellular carcinoma as shown by ultrasonography and/or computed tomography. Patients with the above criteria were enrolled regardless of whether the serum level of aminotransferase was normal or abnormal. Patients with any of the following criteria were excluded from the study: 1) illness that could seriously reduce their life expectancy, 2) findings suggestive of other chronic liver disease, and 3) refusal to be followed up after the diagnosis of NAFLD. A total of 3,559 out of 9,120 patients were excluded based on the following findings; 169 had a dipstick-positive proteinuria; 1,685 had an eGFR of <60 mL/min/1.73 m^2; 2,098 had alcohol intake of ≥20 g/day; 133 had positive serologic findings for either hepatitis B or C virus, a reported history of known liver disease, or decompensated liver cirrhosis; 36 had a history of malignancy; 26 had a history of cardiovascular disease; 11 refused the participation of prospective follow-up. Because some individuals were excluded for multiple reasons, the total number of eligible patients for the study was 5,561.

Patients were classified into three groups according to fasting plasma glucose (FPG): 1) those with FPG level of < 110 mg/dL (normal glucose group), 2) those with FPG level of 110-125 mg/dL (pre-diabetes group), and 3) those with FPG level of ≥126 mg/dL (diabetes group) (25). Patients were regarded as hypertension by the confirmation of blood pressure ≥140 mmHg systolic and/or ≥90 mmHg diastolic.

The primary goal was the new onset of CKD in patients with NAFLD. The end-point was defined as the first eGFR <60 mL/min/1.73 m^2 or dipstick proteinuria (≥ +1) for more than three months. Serum creatinine level was also measured using an enzymatic method, and the GFR was estimated from the Japanese Society of Nephrology CKD Practice Guide; eGFR (mL/min/1.73 m^2) =194× (serum creatinine level [mg/dL]) -1.094× (age [y]) -0.287. The product of this equation was multiplied by a correction factor of 0.739 for women. CKD and its stages were defined from estimated eGFR of <60 mL/min/1.73 m^2 or dipstick proteinuria (≥ +1) as follows: stage I, eGFR≥90 and proteinuria (≥ +1); stage II, 90>eGFR≥60 and proteinuria (≥ +1); stage III, 60>eGFR≥30; stage IV, 30>eGFR≥15; and stage V, 15>eGFR. Patients with stage III-V were regarded as having CKD regardless of the absence of other markers of kidney damage (21, 22).

All of the studies were performed retrospectively by collecting and analyzing data from the patient records. This study was approved by Institutional Review Board of our hospital.

Medical evaluation

Fatty liver was diagnosed by the presence of an ultrasonographic pattern consistent with bright liver with stronger echoes in the hepatic parenchyma than in the renal or spleen parenchyma (26). Ultrasonography test was performed with a high-resolution, real-time scanner (model SSD-2000; Aloka Co., Ltd, Tokyo Japan. Mode Logic-700 MR; GE-Yokokawa Medical Systems, Tokyo, Japan). Body weight was measured in light clothing and without shoes to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm. Height and weight were recorded at baseline and the body mass index (BMI) was calculated as weight (in kg) / height (in m^2). All of the patients were interviewed in the Toranomon Hospital using a questionnaire that gathered information on demographic characteristics, medical history, and heath-related habits including questions on alcohol intake at the time of diagnosis of fatty liver.

Laboratory investigation

At the first consultation anti-HCV and HBsAg were examined. Anti-HCV was detected using a third-generation enzyme-linked immunosorbent assay (Abbott Laboratories, North Chicago, IL). HBsAg was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). Anti-HBs was not evaluated in the present study. Serum creatinine concentration was measured by a modified Jaffe method (creatinine

Table 1. Characteristics of Subjects Enrolled

	Total
Number of cases	5561
Age (years)	48.0±8.4
Sex(male/female)	4916/645
Systolic blood pressure(mmHg)	134±18
Diastolic blood pressure(mmHg)	76±10
Hypertension(+)	725(13.0%)
Height(cm)	167.8±7.3
Body Weight (kg)	70.7±9.9
BMI (kg/m^2)	25.1±2.8
Smoking (+)	1028 (18.5%)
FPG(mg/dL)	104.7±24.8
Glucose status (Normal/ preDM/DM)	4436(79.8%)/667(12.0%)/458(8.2%)
eGFR (mL/min/1.73m^2)	74.6±11.9
WBC(×10^3/mm^3)	5.8±1.5
Hemoglobin (g/dL)	15.1±1.2
Platelet (×10^4/mm^3)	23.1±5.0
Triglyceride (mg/dL)	164±117
Total cholesterol (mg/dL)	210±34
HDL cholesterol (mg/dL)	48.1±11.9
Total Protein(g/dL)	7.5±0.4
Albumin (g/dL)	4.2±0.3
Uric Acid (mg/dL)	6.2±1.3
AST (IU/L)	29.2±16.4
ALT (IU/L)	37.5±27.0
GGT(IU/L)	78.2±81.0
Follow-up period (years)	5.5±4.8

Data are number of patients (percent) or mean ± standard deviation
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DM, diabetes mellitus, eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, gamma-glutamyltransferase; HDL, high density lipoprotein; WBC, white blood cell;

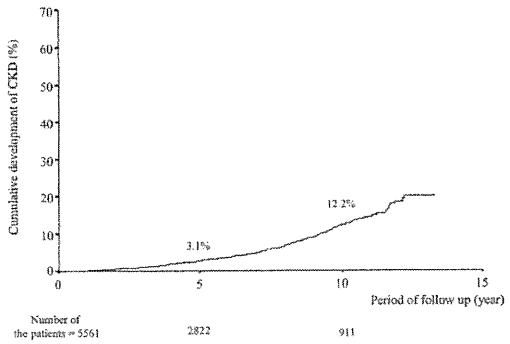

Figure 1. Cumulative development rate of CKD in 5,561 patients with NAFLD.

HR, Wako Pure Chemicals Industries, Ltd, Osaka, Japan) using an autoanalyzer (Hitachi 7350, Hitachi Ltd, Tokyo, Japan or RX-20, JEOL Ltd., Tokyo, Japan). Proteinuria was tested using dipsticks (Ames Hemacombistics; Bayer-Sankyo Ltd, Tokyo, Japan). A test result of ≥ +1 was defined as positive.

Follow-up

Starting time of follow-up was the day that the fatty liver was confirmed by ultrasonography. After that, patients were followed up monthly to six-monthly in the Toranomon hospital. A blood sample and a urine sample were taken for routine analyses. Four hundred and ninety-two patients were lost to follow-up. Because the appearance of CKD was not identified in these 492 patients, they were considered as censored data in statistical analysis (27).

Statistical Analysis

The cumulative appearance rate of CKD was calculated from the starting time of follow-up to the development of CKD by using the Kaplan-Meier method. Differences in the development of CKD were tested using the log rank test. The Cox proportional hazard model analyzed independent factors associated with the development rate of CKD. The following variables were analyzed for potential covariates for incidence of CKD: age, BMI, T2DM, hypertension, and levels of eGFR, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), total protein, triglyceride (TG), total cholesterol level, high density lipoprotein (HDL) cholesterol uric acid, hemoglobin, white blood cell, platelet at the time of diagnosis of NAFLD. A p value of less than 0.05 was considered significant. Data analysis was performed using the computer program SPSS package (SPSS 11.5 for Windows, SPSS, Chicago, IL).

Results

Patients' characteristics

Table 1 shows the characteristics in the 5,561 patients diagnosed as NAFLD in the present study. The mean age was 48 years. The mean BMI was 25.1. Patients with hypertension accounted for 13.0% and patients with T2DM accounted for 8.2%. The eGFR level was 74.6±11.9 mL/min/1.73 m^2. The mean follow-up period was 5.5 years.

Incidence of CKD in Patients with NAFLD

Of 5,561 NAFLD patients, 263 developed CKD. Figure 1 shows that the cumulative development rate of CKD was 3.1% at the 5th year and 12.2% at the 10th year in all patients with NAFLD. Cox proportional hazards analysis showed that CKD development in NAFLD patients occurred when patient had eGFR of 60-75 mL/min/1.73 m^2 [hazard ratio:2.75; 95% confidence interval (CI) =1.93-3.94; p< 0.001], age of ≥50 years (hazard ratio:2.67; 95% CI =2.06-3.46; p<0.001), T2DM (hazard ratio:1.92; 95% CI=1.45-2.54; p<0.001), hypertension (hazard ratio:1.69; 95% CI= 1.25-2.29; p<0.001), and elevated serum GGT (hazard ratio: 1.35; 95% CI=1.02-1.78; p=0.038) at the initiation of follow up (Table 2).

Figure 2 shows the cumulative development rate of CKD based on the difference of age and eGFR level at the starting time of follow-up. Figure 3 shows the cumulative development rate of CKD based on the difference of FPG, blood pressure, and serum GGT at the starting time of follow-up. On the difference of serum GGT level, the cumulative rate of CKD at 10th year in NAFLD was 11.3% in patients with

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Amino Acid Substitutions in Hepatitis C Virus Core Region Predict Hepatocarcinogenesis Following Eradication of HCV RNA by Antiviral Therapy

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Substitution of amino acid (aa) 70 and/or 91 in the core region of HCV genotype 1b (HCV-1b) is an important predictor of hepatocarcinogenesis, but its impact on the development of hepatocellular carcinoma (HCC) following eradication of HCV RNA by antiviral therapy is not clear. 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of interferon monotherapy or interferon plus ribavirin combination therapy, were included in a follow-up study to evaluate the impact of aa substitution in the core region on hepatocarcinogenesis. Twenty six patients developed HCC during the follow-up. The cumulative rates of new HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively. The rates in patients infected with HCV-1b/Gln70(His70) [glutamine (histidine) at aa 70] were significantly higher than in patients infected with HCV-1b/Arg70 (arginine at aa 70) ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). The rates in patients infected with HCV-1b/Arg70 were not significantly higher than in those infected with HCV-2a/2b ($P = 0.617$; log-rank test). Multivariate analysis identified HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced fibrosis (HR 9.03, $P = 0.002$), and old age (HR 3.09, $P = 0.066$) as determinants of hepatocarcinogenesis. In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of HCC following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy. ***J. Med. Virol. 83:1016–1022, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: HCV; genotype; sustained virological response; hepatocellular carcinoma; core region; glutamine

INTRODUCTION

Infection with hepatitis C virus (HCV) is often persistent and can progress to chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. At present, interferon (IFN), in combination with ribavirin, is the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) and high viral loads account for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Tsubota et al., 2005].

Despite numerous lines of epidemiological evidence of an association between HCV infection and the development of HCC, it remains controversial whether the virus itself plays a direct role or an indirect role in the pathogenesis of HCC [Koike, 2005]. It has become evident that the HCV core region is potentially oncogenic in transgenic mice, but the clinical impact of the core region on hepatocarcinogenesis is still unclear [Moriya et al., 1998]. Previous reports indicated that amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b are pretreatment predictors of poor virological response to pegylated IFN (PEG-IFN)/ribavirin combination therapy and triple therapy of

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telaprevir/PEG-IFN/ribavirin [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also affect hepatocarcinogenesis [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. These reports support the oncogenic potential of the core region from the clinical aspect. However, hepatocarcinogenesis still occurs even after eradication of HCV RNA by antiviral therapy [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008], though whether substitutions of aa 70 and/or 91 in the core region also affect hepatocarcinogenesis following eradication of HCV RNA await further investigation.

The present study included 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy). The aims of this study were to evaluate the impact of aa substitutions in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

PATIENTS AND METHODS

Patients

Among 4,570 consecutive patients infected with HCV, in whom antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy) was initiated between February 1987 and June 2010 at the Toranomon Hospital, 1,273 were selected for the present study. We included patients who fulfilled the following criteria: (1) Patients positive for anti-HCV (by a third-generation enzyme immunoassay, Chiron Corp., Emerville, CA) and for HCV RNA by qualitative or quantitative analysis, before antiviral therapy. (2) Patients with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy, based on HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim, Germany) or by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (3) Patients without HCC, before and during IFN therapy. (4) Patients infected with a single genotype of HCV-1b, 2a, or 2b. (5) Patients negative for hepatitis B surface antigen (by radioimmunoassay, Dainabot, Tokyo). (6) Patients free of coinfection with the human immunodeficiency virus. (7) Lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake). (8) Patients free of other types of hepatitis, and without hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) Each signed a consent form of the study protocol that had been approved by the human ethics review committee.

Table I summarizes the profile and laboratory data at the start of antiviral therapy of 1,273 patients with sustained virological response. They included 783 males and 490 females, aged 15–83 years (median, 53 years). The median follow-up time, from the end of antiviral therapy until the last visit, was 1.1 years (range, 0.0–18.0 years).

Laboratory Investigations

Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [Chayama et al., 1993]. HCV RNA was quantitated by branched DNA assay version 2.0 (Chiron Corp.), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems, Inc., Pleasanton, CA), or COBAS TaqMan HCV test (Roche Diagnostics). A high viral load was defined as branched DNA assay value of

TABLE I. Clinical Profile and Laboratory Data at the Start of Antiviral Therapy

Demographic data	
Number of patients	1,273
Sex (male/female)	783/490
Age (years)*	53 (15–83)
Body mass index (kg/m^2)*	22.7 (14.4–38.0)
Laboratory data	
Serum aspartate aminotransferase (IU/L)*	48 (11–1,386)
Serum alanine aminotransferase (IU/L)*	68 (10–2,009)
Total cholesterol (mg/dl)*	168 (79–328)
Fasting plasma glucose (mg/dl)*	93 (69–290)
HCV genotype (1b/2a/2b)*	664/433/176
Level of viremia (high viral load/low viral load)	838/415
Treatment regimen	
IFN monotherapy/IFN plus ribavirin	545/728
Histological findings	
Stage of fibrosis (F1/F2/F3/F4)	508/224/62/47
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 [arginine/glutamine (histidine)]	348/127
Core aa 91 (leucine/methionine)	321/156

The enrolled patients had sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy.
Data are numbers and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

≥1.0 Meq/ml, AMPLICOR GT HCV Monitor ≥100 × 10^3 IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay value of <1.0 Meq/ml, AMPLICOR GT HCV Monitor <100 × 10^3 IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim) was 100 copies/ml, and that of COBAS TaqMan HCV test was 1.2 log IU/ml. Samples with undetectable HCV RNA at 24 weeks after cessation of antiviral therapy by qualitative analysis or COBAS TaqMan HCV test were defined as HCV RNA-negative.

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

In the present study, aa substitutions in the core region of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of antiviral therapy and reverse transcribed with a random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids of the core region were amplified by nested PCR using the following primers. The first-round PCR was performed with CE1 (sense, 5′-GTC TGC GGA ACC GGT GAG TA-3′, nucleotides: 134–153) and CE2 (antisense, 5′-GAC GTG GCG TCG TAT TGT CG-3′, nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense, 5′-ACT GCT AGC CGA GTA GTG TT-3′, nucleotides: 234–253) and CE6 (antisense, 5′-GGA GCA GTC GTT CGT GAC AT-3′, nucleotides: 934–953) primers. All samples were initially denatured at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

Using HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed using 50 clinical samples 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]. Thus, patients were classified into three HCV subgroups according to the HCV genotype and aa substitutions in the HCV-1b core region: (1) HCV-1b with Arg70, (2) HCV-1b with Gln70(His70), and (3) HCV-2a/2b.

Liver Histopathological Examination

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan). The samples were fixed in 10% formalin and then stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. Each specimen submitted for examination contained ≥6 portal areas. Histopathological diagnosis was made by an experienced liver pathologist (HK) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the scoring system of Desmet et al. [1994] for histopathological assessment.

Follow-Up and Diagnosis of Hepatocellular Carcinoma

Hematological, biochemical, and virological tests were performed at least once every month until the virological response was determined. When sustained virological response was confirmed, blood tests and imaging studies (computed tomography or ultrasonography) were conducted once or twice per year in the majority of patients, except those lost to follow-up. When HCC was suspected, additional procedures, such as magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy when necessary, were used to confirm the diagnosis.

Statistical Analysis

The cumulative rate of new cases of HCC was calculated using the Kaplan–Meier technique, and differences between the curves were tested using the log-rank test. Differences in the proportion of new cases of HCC according to groups were analyzed according to the period between the end of antiviral therapy and appearance of HCC. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with the development of HCC. The hazard ratio (HR) and 95% confidence interval (95%CI) were also calculated. Potential predictive factors associated with the development of HCC included the following variables: sex, age, body mass index, AST, ALT, total cholesterol, fasting plasma glucose, HCV genotype, level of viremia, treatment regimen, stage of fibrosis, and HCV subgroup according to HCV genotype in combination with aa substitutions in the core region. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL). All P values of less than 0.05 by the two-tailed test were considered significant.

RESULTS

Rate of New Cases of HCC in Patients With Sustained Virological Response

During the follow-up, 26 patients (2.0%) developed HCC. The median interval between the end of antiviral therapy and detection of HCC (latency to HCC) was 2.5 years (range, 0.0–15.9 years). The cumulative rates of new cases of HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively.

HCC Rate According to HCV Genotype and Amino Acid Substitutions in the Core Region of HCV-1b

During the follow-up, 7 (5.5%), 5 (1.4%), and 12 (2.0%) patients developed HCC in the HCV-1b with Gln70(His70), HCV-1b with Arg70, and HCV-2a/2b groups, respectively. The median latency to HCC was 1.1 years (range, 0.0–14.0 years), 3.9 (range, 0.0–15.9), and 2.8 (range, 0.0–12.9), respectively, and the cumulative rates of new cases of HCC were 10.6%, 3.6%, 3.0% at the end of 5 years; 10.6%, 6.3%, 5.2% at the end of 10 years; and 62.7%, 6.3%, 7.2% at the end of 15 years, respectively. The rates were significantly different among the three HCV subgroups ($P < 0.001$; log-rank test; Fig. 1). Especially, the rates for HCV-1b with Gln70(His70) were significantly higher than those for HCV-1b with Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). However, the rates for the HCV-1b with Arg70 group were not significantly higher than those for the HCV-2a/2b group ($P = 0.617$; log-rank test).

During the follow-up, 4 (2.6%) and 7 (2.2%) patients with HCV-1b/Met91, and HCV-1b/Leu91 developed HCC, respectively. In these two subgroups, the respective median latency to HCC was 3.4 years (range, 0.0–14.0 years) and 1.1 (range, 0.0–12.4), and the cumulative rates of new cases of HCC were 1.3%, 8.6% at the end of 5 years; 5.4%, 8.6% at the end of 10 years; and 36.9%, 14.7% at the end of 15 years. The rates for the HCV-1b/Met91 group were not significantly different from those for the HCV-1b/Leu91 group ($P = 0.908$; log-rank test).

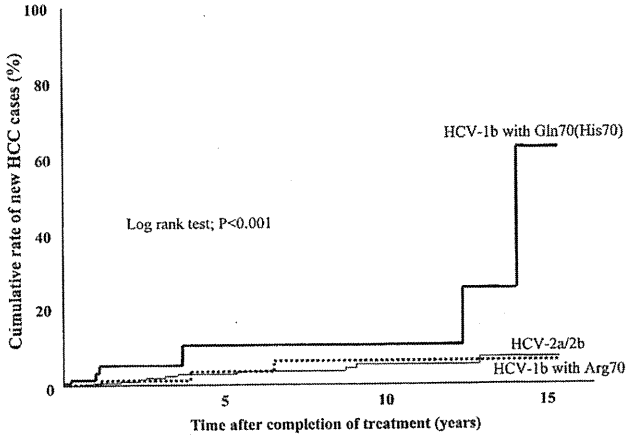

Fig. 1. Cumulative rates of new cases of HCC according to HCV genotype and amino acid substitutions in the core region of HCV-1b. The rates were significantly different among the three HCV groups ($P < 0.001$; log-rank test). Especially, the rate in patients with HCV-1b/Gln70(His70) was significantly higher than those of patients with HCV-1b/Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). Furthermore, the rate in patients with HCV-1b/Arg70 was not significantly higher than that in HCV-2a/2b ($P = 0.617$; log-rank test).

Predictive Factors Associated With the Development of HCC in Patients of Sustained Virological Response

Next, we analyzed the predictor of HCC using data of the entire group. There were significant relationships between the rate of new cases of HCC and male sex ($P = 0.003$), severe fibrosis (F3,4) ($P < 0.001$), old age (≥55 years) ($P = 0.002$), high levels of AST (≥39 IU/L) ($P = 0.023$), and HCV-1b/Gln70(His70) (log-rank test). These five factors were entered into multivariate analysis, which then identified three parameters that independently tended to or significantly influenced the development of HCC; HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced stage of fibrosis (F3,4; HR 9.03, $P = 0.002$), and old age (≥55 years; HR 3.09, $P = 0.066$; Table II).

Predictors of HCC in HCV-1b Patients With Sustained Virological Response

Finally we analyzed the data of 664 patients with HCV-1b to determine the predictors of HCC with sustained virological response. Univariate analysis identified three parameters that significantly correlated with the development of HCC: male sex ($P = 0.005$), old age ($P = 0.020$), and HCV-1b with Gln70(His70) ($P = 0.007$; log-rank test). These three factors were entered into multivariate analysis, which then identified HCV-1b with Gln70(His70) as the single parameter that significantly influenced the development of HCC (HR 8.19, $P = 0.034$).

DISCUSSION

Previous studies reported that the risk factors for hepatocarcinogenesis after elimination of HCV RNA

TABLE II. Results of Multivariate Analysis (Cox Proportional Hazard Model) for Factors Associated With Hepatocarcinogenesis in Patients With Sustained Virological Response

Factors and categories	Hazard ratio (95%CI)	*P*-Value
HCV group		
HCV-2a/2b	1	
HCV-1b with Arg70	1.15 (0.24–5.56)	0.863
HCV-1b with Gln70(His70)	10.5 (2.89–38.2)	<0.001
Fibrosis stage		
F1,2	1	
F3,4	9.03 (2.32–35.2)	0.002
Age (years)		
<55	1	
≥55	3.09 (0.93–10.3)	0.066

were severe fibrosis, male sex, and old age at the start of IFN treatment [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008]. In the present study, multivariate analysis identified HCV-1b with Gln70(His70), advanced fibrosis stage, and old age as determinants of HCC in patients with a sustained virological response. The present study is the first report to indicate that aa substitution in the core region at the start of antiviral therapy also influences hepatocarcinogenesis following eradication of HCV RNA. This result should be interpreted with caution since races other than the Japanese and patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies of patients of other races and those infected with HCV-1a.

Despite numerous lines of epidemiological evidence linking HCV infection to the development of HCC, it remains controversial whether HCV itself plays a direct or indirect role in the pathogenesis of HCC [Koike, 2005]. Evidence suggests that the HCV core region is potentially oncogenic in the transgenic mice [Moriya et al., 1998], though the clinical impact of the core region on hepatocarcinogenesis remains unclear. Previous reports indicated that aa substitutions in the core region of HCV-1b are pretreatment predictors of poor virological response to antiviral therapy [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also are etiological factors in HCC [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. Importantly, the present study indicated that aa substitution in the core region at the start of antiviral therapy also affects the development of HCC even after the eradication of HCV RNA, and this is the first report to suggest the persistent oncogenic potential of the core region regardless of HCV RNA persistence. Previous reports identified the PA28γ-dependent pathway as one of the mechanisms of HCV-associated hepatocarcinogenesis. Moriishi et al. [2003, 2007] reported that knockout of the PA28γ gene induces accumulation of HCV core protein in the nuclei of hepatocytes of HCV core gene transgenic mice and disrupts the development of both hepatic steatosis and HCC. Furthermore, the HCV core protein also enhances the binding of liver X receptor α (LXRα) and retinoid X receptor α (RXRα) to the LXR-response element in the presence of PA28γ [Moriishi et al., 2007]. Thus, it seems that PA28γ plays a crucial role in the development of HCV-associated steatosis and HCC. However, these basic studies were performed under the state of HCV RNA persistence [Moriya et al., 1998; Moriishi et al., 2003, 2007; Koike, 2005], and further studies should be performed to investigate the oncogenic potential of aa substitution in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

The association between HCV genotype and the risk of HCC is not clear. A study of Italian cohort indicated that the rate of HCC in patients infected with HCV-1b was significantly higher than that of patients infected with HCV-2a/2c [Bruno et al., 2007]. On the other hand, the present study of Japanese patients indicated that the rates in patients infected with HCV-1b were not significantly higher than those in those infected with HCV-2a/2b. The discrepancy between the present result and the above Italian study may be explained by differences in host factors [Montes-Cano et al., 2010], and/or differences in viral factors, such as the distribution of HCV-1b with Arg70 or Gln70(His70), and geographic diversities of HCV-1b [Nakano et al., 1999].

Previous studies showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin achieved sustained virological response rates of 35–60% and 61–69% in patients infected with HCV-1, respectively [Hézode et al., 2009; McHutchison et al., 2009; Akuta et al., 2010]. Furthermore, the PROVE3 study also showed that the 24- and 48-week regimen of triple therapy achieved sustained virological response rates of 51% and 53%, respectively, in patients infected with HCV-1 who had been unsuccessfully treated with PEG-IFN/ribavirin [McHutchison et al., 2010]. While it is anticipated that larger numbers of HCV-1 patients will achieve sustained virological response in response to telaprevir/PEG-IFN/ribavirin, a larger proportion of patients could develop HCC following eradication of HCV RNA by antiviral therapy. Hence, our study indicated that aa substitutions in the core region of HCV-1b should be detected before eradication of HCV RNA by antiviral therapy. Especially, even if patients of HCV-1b with Gln70(His70) could achieve sustained virological response, blood tests and imaging studies should be conducted at regular intervals in this high risk group for early detection and treatment of HCC.

Genetic variations near the IL28B gene are pretreatment predictors of poor virological response to the combination therapy of PEG-IFN/ribavirin and triple therapy of telaprevir/PEG-IFN/ribavirin [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Akuta et al., 2010; Rauch et al., 2010], but their impact on hepatocarcinogenesis are unknown at this stage. In this study, 387 of 1,273 patients were evaluated for HCC according to genetic variation in rs8099917 (data not shown). A preliminary study based on a small number of patients showed that the HCC rate in genotype TT of treatment sensitive type (2.2%) was not significantly different from that in genotype non-TT of treatment resistant type (1.6%). Unfortunately, we could analyze the effect of rs8099917 on HCC following eradication of HCV RNA by antiviral therapy. Further studies of larger patient populations should be performed to investigate the relationship between genetic variations near the IL28B gene and HCC.

The limitations of the present study were that viral factors associated with hepatocarcinogenesis were incompletely investigated. Ogata et al. [2003] reported that HCV-1b strains might be associated with HCC

on the basis of the secondary structure of the amino-terminal portion of the HCV NS3 protein. Giménez-Barcons et al. [2001] reported that high amino acid variability within the NS5A of HCV might be associated with HCC in patients with HCV-1b-related cirrhosis. In the present study, the clinical impact of other regions on hepatocarcinogenesis could not be investigated, except for aa 70 and 91 in the HCV core region. The results should also be interpreted with caution since patients infected with HCV-1a were not included. Other limitations include lack of analysis of the effects of life-style related diseases (such as diabetes, insulin resistance or non-alcoholic steatohepatitis) on hepatocarcinogenesis, except for fasting plasma glucose and total cholesterol [Sumida et al., 2010a,b]. The impact of viral factors and life-style related diseases on hepatocarcinogenesis should also be investigated in future studies.

In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of hepatocarcinogenesis following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy.

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