derived from the Hepatitis C Antiviral Long-term Treatment Against Cirrhosis (HALT-C) Trial identified older age, African American race, lower platelet count, higher alkaline phosphatase, and esophageal varices as risk factors for HCC [11].

There have also been a number of studies to evaluate the effect of anti-viral treatment of chronic hepatitis C on the incidence of HCC [12–19]. The results were summarized in a meta-analysis, which concluded that the effect of interferon on risk of HCC is mainly apparent in patients achieving a sustained virological response (SVR) to interferon therapy [13]. In addition, a number of studies have suggested the incidence of HCC is reduced in treated patients compared to historical controls [12, 15, 16, 19]. However, the recent HALT-C randomized control trial revealed that long-term pegylated interferon therapy does not reduce the incidence of HCC among patients with advanced hepatitis C who do not achieve SVRs. Reduction in the risk of HCC by maintenance therapy was shown only in patients with cirrhosis [14, 17]. These controversial results suggest that interferon therapy reduces the risk of HCC only in a group of patients with HCV-related chronic liver disease. Thus, it is important to evaluate the risk of HCC development in hepatitis C patients receiving interferon therapy and it will be clinically useful to discover markers distinguishing high- and low-risk groups.

Serum alpha-fetoprotein (AFP) has been widely used as a diagnostic marker of HCC [20–22]. However, elevation of serum AFP levels is often found in non-neoplastic liver diseases without evidence of HCC, including acute liver injury and chronic viral hepatitis [23–27], especially among patients with advanced chronic hepatitis C [28]. An increase of AFP after liver damage is interpreted as a sign of dedifferentiated hepatic regeneration [27]. There have been some reports that AFP is a significant predictor of HCC in patients with chronic hepatitis C [4, 5, 29]. In addition, it has recently been shown that AFP levels decrease in response to interferon administration in patients with chronic hepatitis C [30, 31], and that long-term interferon therapy for aged patients with chronic HCV infection is effective in decreasing serum AFP levels and preventing hepatocarcinogenesis [32, 33]. However, little is known about the relationship between changes in serum AFP level over time during interferon therapy and the development of HCC.

The aim of this large single center study was to identify predictive markers for the risk of HCC development in patients receiving interferon therapy for chronic hepatitis C. For this purpose, patients treated with standard or pegylated interferon, in combination with ribavirin, for chronic hepatitis C were enrolled and subjected to scheduled periodic surveillance for HCC and a number of potential predictive markers, including AFP and alanine aminotransferase (ALT) integration values, at a single center.

Materials and methods

Patients

Between January 2002 and April 2010, 528 patients with chronic hepatitis C received combination therapy with standard interferon and ribavirin ($n = 84$) or pegylated interferon and ribavirin ($n = 444$) at Osaka Red Cross Hospital. Eligibility criteria for treatment were positivity for serum HCV RNA and histological evidence of chronic hepatitis C ($n = 427/444$; 80.9%), or positivity for serum HCV RNA, liver enzyme levels greater than the normal upper limit, and an ultrasound image demonstrating chronic liver damage ($n = 101/444$; 19.1%). Exclusion criteria for treatment were as follows: neutrophil count <750 cells/μL, platelet count <50,000 cells/μL, hemoglobin level ≤9.0 g/dL, and renal insufficiency (serum creatinine levels >2 mg/dL).

Of 528 patients who received interferon therapy for chronic hepatitis C, 146 were excluded from this study for the following reasons: follow-up <24 weeks after the termination of the interferon therapy ($n = 122$), previously treated for HCC ($n = 22$), or occurrence of HCC during or within 24 weeks after treatment ($n = 2$). Therefore, 382 patients were enrolled for the study and were retrospectively analyzed.

To detect early-stage HCC, ultrasonography, dynamic contrast enhanced computed tomography (CT), dynamic contrast enhanced magnetic resonance imaging (MRI), and/or measurement of tumor markers (including AFP) were performed for all patients at least every 6 months. HCC was diagnosed radiologically as liver tumors displaying arterial hypervascularity and venous or delayed phase washout by dynamic contrast enhanced CT or MRI.

The study protocol was approved by the Ethics Committee at Osaka Red Cross Hospital and performed in compliance with the Helsinki Declaration.

Treatment protocol and definition of responses to treatment

The basic treatment protocol for patients with chronic hepatitis C consisted of 6 mega units of interferon-α-2b 3 times a week or 1.5 μg/kg of pegylated interferon α-2b once a week, combined with ribavirin at an oral dosage of 600–1000 mg/day. Duration of the treatment was 48–72 weeks for those with HCV genotype 1 and serum HCV RNA titer of >5 log IU/mL, and 24 weeks for all other patients.

Patients who were negative for serum HCV RNA for >6 months after completion of interferon therapy were defined as showing an SVR. Patients whose serum ALT levels decreased to the normal range and remained normal for >6 months after the termination of interferon therapy were defined as showing a sustained biochemical response (SBR).

Patients who did not achieve SVR received ursodeoxycholic acid and/or glycyrrhizin containing preparation (Stronger Neo-Minophagen C), when serum ALT levels were higher than the upper limit of normal.

Virological assays

HCV genotype was determined by polymerase chain reaction (PCR) amplification of the core region of the HCV genome using genotype-specific PCR primers [34]. Serum HCV RNA load was evaluated once a month during and 24 weeks after treatment using a PCR assay (Cobas Amplicor HCV Monitor, Roche Molecular Systems, Pleasanton, CA, USA).

Measurement of AFP and calculation of average integration value

AFP was measured in serum samples obtained from each patient at intervals of 1–3 months. The median number of examinations was 15 (range 1–70) in each patient. Serum AFP levels were determined by enzyme-linked immunosorbent assay, which was performed using a commercially available kit (ELISA-AFP, International Reagents, Kobe, Japan). Integration values of AFP and ALT were calculated as described in previous reports [35]. For example, the integration value of AFP was calculated as follows, $(y_0 + y_1) \times x_1/2 + (y_1 + y_2) \times x_2/2 + (y_2 + y_3) \times x_3/2 + (y_3 + y_4) \times x_4/2 + (y_4 + y_5) \times x_5/2 + (y_5 + y_6) \times x_6/2$, i.e., the area of each trapezoid representing an AFP value was measured the sum of the resulting values used to calculate the integration value (Fig. 1). The average integration value was obtained by dividing the integration value by the observation period from initiation of the treatment.

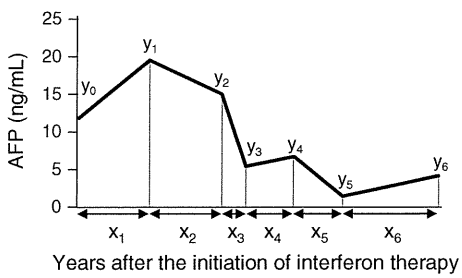

Fig. 1 Example plot of data used for calculation of average integration value of alpha-fetoprotein (AFP)

Statistical analysis

The Kaplan–Meier method was used to estimate the rates of development of HCC in patients after interferon therapy. Log-rank tests were used to evaluate the effects of predictive factors on incidence of HCC. Significance was defined as $P < 0.05$. Multivariate Cox regression analysis using the stepwise method was used to evaluate the association between HCC incidence and patient characteristics, and to estimate hazard ratio (HR) with a 95% confidence interval (CI). A P value of 0.1 was used for variable selection and was regarded as statistically significant. SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Characteristics of patients and incidence of HCC

This study included 382 patients treated for chronic hepatitis C with standard interferon or pegylated interferon in combination with ribavirin. Baseline clinical and virological characteristics of patients included in the study are summarized in Table 1. The median age of the patients at the outset of therapy was 59.0 years (range 18–81 years) and the median follow-up period was 4.1 years (range 0.1–8.4 years). The majority of patients were infected with HCV genotype 1b ($n = 229$; 60%), and median serum HCV RNA load was 6.1 log IU/mL (range 2.3–7.3 log IU/mL). Baseline (before interferon therapy) median serum AFP level was 6.9 ng/mL (range 1.6–478.3 ng/mL).

During follow-up, 23 patients (4.9%) developed HCC. The cumulative incidences of HCC, which was estimated using the Kaplan–Meier method, were 3.1, 6.6, and 13.4% at 3, 5, and 8 years, respectively (Fig. 2).

Predictive factors for incidence of HCC in all patients

Predictive factors for incidence of HCC in all 382 patients were analyzed using log-rank tests (Table 2). Univariate analysis showed that age ≥70 years ($P = 0.040$), non-SVR ($P < 0.0001$), non-SBR ($P = 0.027$), average ALT integration value ≥40 IU/L ($P = 0.001$), baseline AFP ≥10 ng/mL ($P = 0.005$), average AFP integration value ≥10 ng/mL ($P < 0.0001$), and baseline platelet count <150,000 platelets/μL ($P = 0.001$) were all significantly associated with the incidence of HCC. After multivariate analysis, the only variable remaining in the model was non-SVR (HR 8.413, 95% CI 1.068–66.300, $P = 0.043$).

Table 1 Characteristics of 382 patients with hepatitis C treated with interferon therapy in this study

Age (years)	59.0 (18–81)
[a]Males/females	192/190
Observation period (years)	4.1 (0.1–8.4)
[a]IFN + RBV/PEG-IFN + RBV	69/313
HCV genotype 1/2/unclassified	229/57/96
HCV RNA (log IU/mL)	6.1 (2.3–7.3)
White blood cell count (/μL)	4950 (2050–9970)
Hemoglobin (g/dL)	14.0 (10.3–18.8)
Platelet (10^4/μL)	15.0 (5.3–36.4)
AST (IU/L)	56 (17–244)
ALT (IU/L)	67 (16–416)
Bilirubin (mg/dL)	0.8 (0.3–2.4)
AFP (ng/mL)	6.9 (1.6–478.3)

Qualitative variables ([a]) are shown in number, and quantitative variables expressed as median (range)

IFN interferon, *RBV* ribavirin, *PEG-IFN* pegylated interferon, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *AFP* alpha-fetoprotein

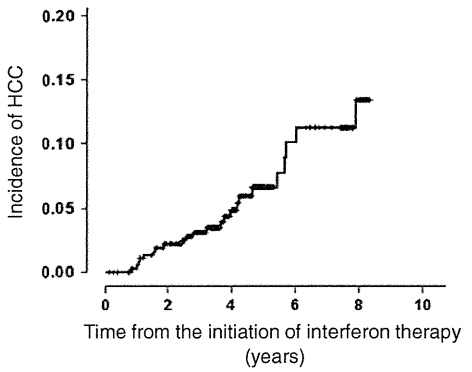

Fig. 2 Incidence of hepatocellular carcinoma (HCC) in 382 patients with hepatitis C who received interferon therapy, estimated using the Kaplan–Meier method

Further, although patients with average AFP integration values ≥10 ng/mL also appeared to have an increased risk of HCC, the difference did not reach statistical significance in the multivariate analysis ($P = 0.050$) (Table 3).

Predictive factors for incidence of HCC in non-SVR patients

Because non-SVR was the only predictive factor across the entire study cohort, to clarify predictive factors for incidence of HCC within this group, the same variables were further analyzed in non-SVR patients alone. By univariate analysis, average AFP integration value ≥10 ng/mL ($P = 0.019$) and baseline platelet count <150,000 ($P = 0.0022$) (Table 2) were again identified as significant predictive factors for incidence of HCC. In addition, male gender was significantly associated with incidence of HCC in non-SVR patients ($P = 0.022$). Multivariate analysis, however, indicated that only two variables were independently associated with incidence of HCC in non-SVR patients: average AFP integration value ≥10 ng/mL (HR 4.039, 95% CI 1.570–10.392, $P = 0.004$), and male gender

Table 2 Univariate analysis of predictive factors for incidence of hepatocellular carcinoma in all 382 and 197 non-SVR patients

Factors	All ($n = 382$)			Non-SVR ($n = 197$)		
	No.	Incidence of HCC ($n = 23$)		No.	Incidence of HCC ($n = 22$)	
		No. (%)	P value[a]		No. (%)	P value[a]
Age (years)						
<70	359	19 (5)	0.040	182	18 (10)	0.089
≥70	23	4 (17)		15	4 (27)	
Sex						
Female	190	8 (4)	0.125	111	8 (7)	0.022
Male	192	15 (8)		86	14 (16)	
HCV genotype						
1	229	12 (5)	0.452	137	12 (9)	0.796
Non-1	57	1 (2)		10	1 (10)	
Virological response						
SVR	185	1 (1)	<0.0001			
Non-SVR	197	22 (11)				
Biochemical response						
SBR	282	12 (4)	0.027	102	11 (11)	0.857
Non-SBR	86	11 (13)		81	11 (14)	
ALT before IFN therapy						
<40	79	2 (3)	0.274	39	2 (5)	0.319
≥40	301	21 (7)		158	20 (13)	
ALT integration value						
<40	238	6 (3)	0.001	79	5 (6)	0.153
≥40	142	17 (12)		118	17 (14)	
AFP before IFN therapy						
<10	230	7 (3)	0.005	102	7 (7)	0.124
≥10	116	14 (12)		75	13 (17)	
AFP integration value						
<10	258	8 (3)	<0.0001	115	8 (6)	0.019
≥10	63	12 (19)		53	11 (21)	
Platelet before IFN therapy						
<150,000	187	20 (11)	0.001	121	19 (16)	0.022
≥150,000	194	3 (2)		76	3 (4)	

[a] Log-rank test

SVR sustained virological response, *SBR* sustained biochemical response, *ALT* alanine aminotransferase, *IFN* interferon, *AFP* alpha-fetoprotein

Table 3 Multivariate analysis of the predictive factors for incidence of hepatocellular carcinoma in all 382 patients

Factors	Hazard ratio	95% CI	*P* value
Virological response			
SVR	1		
Non-SVR	8.413	1.068–66.300	0.043
AFP integration value			
<10	1		
≥10	2.580	0.999–6.659	0.050

SVR sustained virological response, *IFN* interferon, *AFP* alpha-fetoprotein

Table 4 Multivariate analysis of predictive factors for incidence of hepatocellular carcinoma in 197 non-SVR patients

Factors	Hazard ratio	95% CI	*P* value
AFP integration value			
<10	1		
≥10	4.039	1.570–10.392	0.004
Sex			
Female	1		
Male	3.636	1.383–9.563	0.009

AFP alpha-fetoprotein

(HR 3.636, 95% CI 1.383–9.563, $P = 0.009$) (Table 4). There was no significant difference in other variables including those identified as predictive factors in the entire study population (i.e., age, non-SBR, ALT integration value, AFP before interferon therapy) (Table 2).

AFP integration value as a predictive factor for HCC

Further analysis focused on the AFP integration value as this was the strongest predictive factor for incidence of HCC in non-SVR patients. Of the 382 patients, both baseline and AFP integration values were available for 321. These were divided into four groups: (1) AFP "low–low," (2) AFP "low–high," (3) AFP "high–low," and (4) AFP "high–high," for baseline AFP-average AFP integration values, respectively, where "high" is ≥10 ng/mL and "low" is <10 ng/mL. As shown in Fig. 3a, of the 321 patients, 211 (65.7%) showed baseline AFP levels <10 ng/mL. Of these 211, 207 (98%), were in the AFP low–low group, and only four in the AFP low–high groups. Baseline characteristics, including age, gender, serum HCV-RNA, aspartate aminotransferase (AST), ALT, bilirubin, white blood cell, hemoglobin, platelet, observation periods, and number of times of AFP measurement, were not different between AFP high–low group and high–high group. However, AFP-low group, which is a combination of the low–high and low–low groups, showed significantly lower AST level ($P < 0.00001$), lower ALT level ($P < 0.00001$), higher platelet count ($P < 0.00001$), shorter observation period ($P = 0.01448$), and fewer number of times of AFP examination ($P = 0.00035$), compared to both AFP high–high and AFP high–low group. Six patients (2.8%) with baseline AFP levels <10 ng/mL developed HCC in the follow-up period and none of these patients were among the four low–high group patients. Even in patients with high baseline AFP levels, incidence of HCC was only 3.9% among the AFP high–low group (2 of 51 patients). In contrast, 20.3% of patients in the AFP high–high group developed HCC during the follow-up period.

The incidence rate of HCC in three patient groups, "AFP-low" (a combination of the "low–high" and "low–low" groups),"high–low," and "high–high", was estimated using the Kaplan–Meier method and compared using log-rank tests (Fig. 3b). The rate of HCC incidence was significantly higher in the AFP high–high group compared to both the AFP high–low group and patients with low baseline AFP levels ($P = 0.009$ and 0.001, respectively). There was no significant difference between patients with low baseline AFP levels and the AFP high–low group. The 7-year incidence rate of HCC was 32.3% in the AFP high–high group, compared to only 6.6% in the AFP high–low group, and 8.1% in all patients with low pre-treatment levels.

Discussion

It is well recognized that the most effective strategy for the prevention of HCC development in patients with chronic hepatitis C is likely to be the complete elimination of the HCV infection accompanied by the resultant normalization of liver function [7, 12, 13, 15, 16, 19]. Indeed, we confirmed here that non-SVR is the most significant predictive factor for incidence of HCC in patients receiving interferon therapy for chronic hepatitis C. However, it should be noted that the risk of HCC, even in non-SVR patients, differs between individuals. In the current study, we identified AFP integration value and male gender as independent risk factors for incidence of HCC in non-SVR patients. The incidence of HCC was significantly reduced in individuals with average AFP integration values < 10 ng/mL after interferon therapy, which suggests that the decrease of AFP by interferon therapy lowers the risk of developing HCC. Indeed, even where patients had high baseline AFP levels, incidence of HCC was reduced when the AFP integration value decreased after interferon therapy. Thus, our current findings identify AFP integration value as a useful predictive marker of HCC development in non-SVR patients.

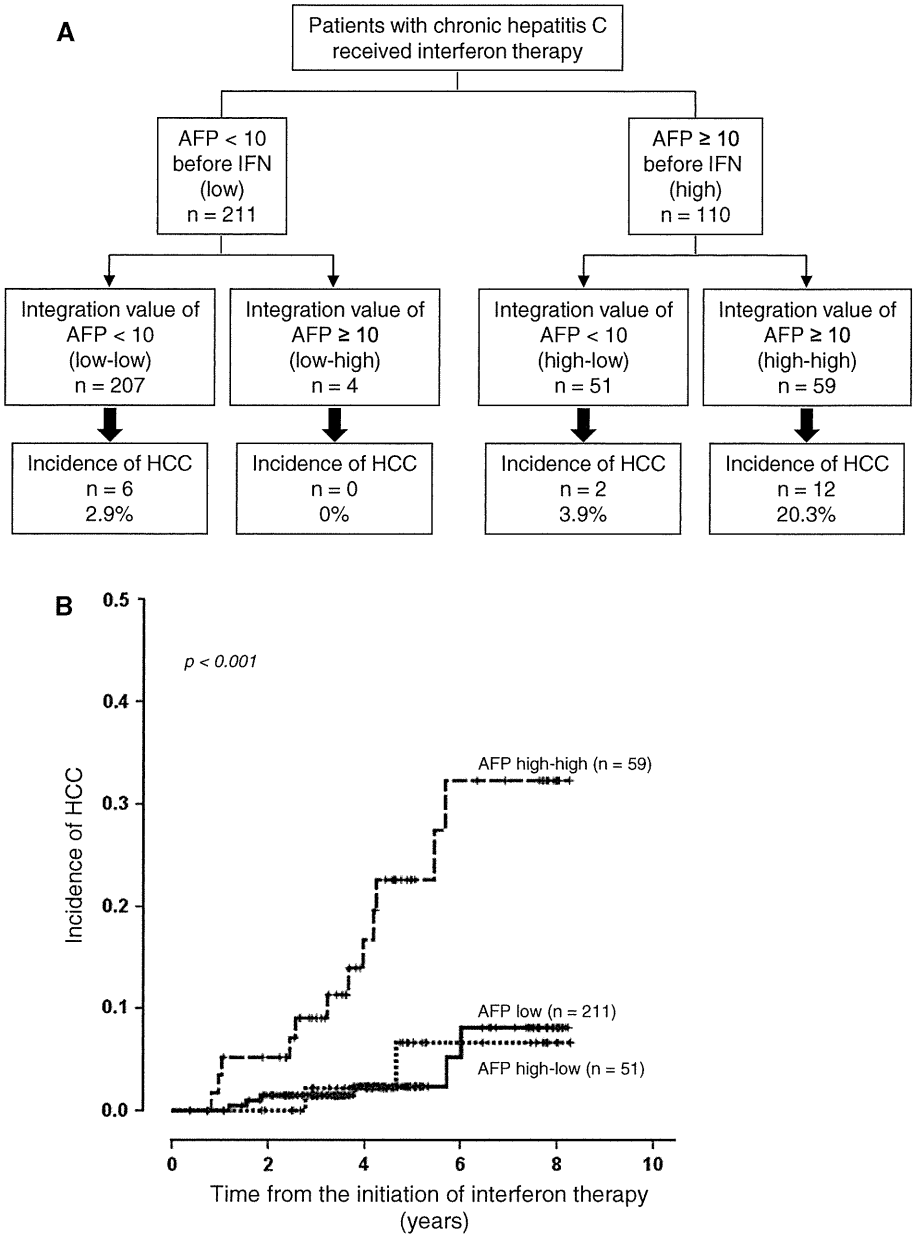

Fig. 3 AFP integration value as a predictive factor for HCC. **a** *Flow diagram* showing the number of patients (*n*) classified by baseline alpha-fetoprotein (AFP) levels before interferon (IFN) therapy and average AFP integration value, and the incidence of hepatocellular carcinoma (HCC) of each group. **b** Kaplan–Meier estimates of the incidence of HCC. *Solid line* AFP-low group (AFP levels before interferon therapy <10 ng/mL); *dotted line* AFP high–low group (baseline AFP levels ≥10 ng/mL, average AFP integration value <10 ng/mL); *dashed line* AFP high–high group (both baseline and average AFP integration values ≥10 ng/mL)

Data from several previous studies suggest that the continuous normalization of alanine aminotransferase (ALT) levels by interferon therapy can reduce the risk of HCC development [36–39]. In addition, one recent study suggested that the ALT integration value is a predictive factor for HCC [35]. In contrast to published data (22), our multivariate analysis did not identify the ALT integration value as a significant predictive factor for HCC incidence, although it was identified as significant by univariate analysis in all 382 patients. Since the previous study did not evaluate AFP levels as a factor for prediction of HCC [35], our results indicate that the AFP integration value is superior to that of ALT as a predictive factor for incidence of HCC. We do not know the reason for this result, but it is speculated that significance of AFP as a marker of hepatic regeneration resulted in the more accurate prediction of hepatocarcinogenesis by integration value of AFP than that of ALT.

As AFP is a diagnostic marker for the existence of HCC, high integration value of AFP in the present study might be a result of HCC development. However, we concluded that the high AFP integration values in patients who developed HCC were not caused by a result of existence of HCC, because of the following two reasons. First, the last AFP values before detection of HCC were not the highest level in the follow-up periods in 19 of 23 patients who developed

HCC, suggesting that the AFP was not produced by the developing HCC in these patients. Second, to exclude the influence of the remaining four patients whose last AFP levels were the highest in the follow-up periods, we analyzed the same statistical analysis by using average AFP integration values excluded the last two examinations of AFP before the detection of HCC. The results of the analysis also showed average integration value of AFP as a significant predictive factor for incidence of HCC.

Male gender was also identified as an independent risk factor for HCC in non-SVR patients in this study. Several reports have shown that men are at a higher risk of developing HCC than women [6, 10, 33, 40, 41]. The male gender also appears to be a risk factor for more severe disease and a greater risk of developing cirrhosis in chronic hepatitis C [42]. Although the association of male gender with the risk of HCC is as yet unexplained, hormonal or genetic factors may lead to increased risk for HCC and cirrhosis in men as previously discussed [10].

In conclusion, a decrease in the AFP integration value predicts reduced incidence of HCC in patients with hepatitis C receiving interferon therapy. Further prospective studies with a larger number of patients are required to validate the significance of these findings.

Acknowledgments This work was supported by Grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor and Welfare of Japan.

Conflict of interest The authors declare that they have no conflict of interest.

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World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i40.5753

World J Gastroenterol 2012 October 28; 18(40): 5753-5758
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Evaluation of sorafenib for hepatocellular carcinoma by contrast-enhanced ultrasonography: A pilot study

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Received: April 19, 2012 Revised: June 18, 2012
Accepted: July 18, 2012
Published online: October 28, 2012

Abstract

AIM: To determine the usefulness of arrival time parametric imaging (AtPI) using contrast-enhanced ultrasonography (CEUS) with Sonazoid in evaluating early response to sorafenib for hepatocellular carcinoma (HCC).

METHODS: Fourteen advanced HCC patients who received sorafenib 400/800 mg/d for at least 4 wk and were followed up by CEUS were enrolled in this study. CEUS was performed before treatment and 2 and 4 wk after treatment, and images of the target lesion in the arterial phase were recorded for each patient. The images were analyzed by AtPI. Color mapping (CM) images obtained by AtPI were compared before and after the treatment. In these CM images, the mean arrival time of the contrast agent in the region of interest from the starting point [mean time (MT)] was calculated. In each patient, differences between MT before and MT 2 and 4 wk after the treatment were compared with responses evaluated 4-8 wk after the treatment by dynamic computed tomography (CT), and statistical analysis was performed. Modified response evaluation criteria in solid tumors was used for the response evaluation.

RESULTS: In CM images both 2 and 4 wk after the treatment, delays in the arrival time of the contrast agent were noted in 8 of the 14 patients. In the other 6 patients, no color changes were observed in the tumor, or red and/or yellow increase, suggesting a decrease in blood flow velocity between images 2 and 4 wk after the treatment and those before the treatment. Dynamic CT could be performed 4-8 wk after the treatment in 13 of the 14 patients. Median differences in the MT were 1.13 s and 1.015 s, 2 and 4 wk after the treatment, respectively, in the 8 patients who showed stable disease (SD)/partial response (PR) on dynamic CT. Median differences in the MT were -0.39 s and -0.95 s, 2 and 4 wk after the treatment, respectively, in the 5 patients who showed progressive disease (PD). Differences in the median MT between SD/PR and PD groups were significant 2 and 4 wk after the treatment with $P = 0.019$ and $P = 0.028$, respectively.

CONCLUSION: AtPI by CEUS using Sonazoid is suggested to be useful for evaluating early responses to sorafenib.

Key words: Sorafenib; Sonazoid; Contrast-enhanced ultrasonography; Hepatocellular carcinoma; Therapeutic response

Peer reviewers: Herwig R Cerwenka, Professor, Department of Surgery, Medical University of Graz, Auenbruggerplatz 29, Graz A-8036, Austria; Bijan Eghtesad, Associate Professor, Department of General Surgery, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, United States; Markus Peck-Radosavljevic, Professor, Department of Gastro-

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Shiozawa K, Watanabe M, Kikuchi Y, Kudo T, Maruyama K, Sumino Y. Evaluation of sorafenib for hepatocellular carcinoma by contrast-enhanced ultrasonography: A pilot study. *World J Gastroenterol* 2012; 18(40): 5753-5758 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i40/5753.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i40.5753

INTRODUCTION

We previously evaluated the hemodynamics of hepatocellular carcinoma (HCC) and metastatic liver tumors based on the time intensity curve (TIC)[1] and arrival time parametric imaging (AtPI)[2,3] employing contrast-enhanced ultrasonography (CEUS) using Sonazoid (Daiichi Sankyo, Tokyo, Japan). As a result, CEUS using Sonazoid has been suggested to allow continuous observation of hemodynamics and is applicable to evaluate the hemodynamics of HCC and the effects of therapeutics on metastatic liver tumors[1,2]. Recently, molecular biological characteristics related to the progression and proliferation of HCC have been clarified, accelerating the development of various molecular-targeting agents[4-6]. Sorafenib[7-9] (Bayer, Leverkusen, Germany) is a multikinase inhibitor that targets multiple molecules, and was approved for use in the treatment of unresectable advanced HCC in Japan in May, 2009. Since it targets tumor growth (RAF-MEK-ERK) and angiogenesis (vascular endothelial growth factor receptor and platelet-derived growth factor receptor) signal transduction pathways, it is expected to be useful for the treatment of HCC[10-14].

The evaluation of chemotherapeutic agents that exert their cytotoxic effects against advanced HCC by inhibiting nucleic acid metabolism has been conducted mainly based upon the tumor volume-reducing effect, according to the response evaluation criteria in solid tumor (RECIST)[15]. The tumor volume-reducing effect of molecular-targeting agents such as sorafenib appears to be slow, although the survival period is considered to be prolonged, even with only a minor volume-reducing effect. Indeed, in the Sorafenib HCC Assessment Randomized Protocol trial in advanced HCC, while the response rate based upon RECIST was only 2%, the survival period in the sorafenib group was significantly longer than in the placebo group, demonstrating its clinical efficacy[10]. Therefore, evaluation of the antineoplastic effect of sorafenib, which exhibits an antitumor effect primarily by inhibiting angiogenesis, is difficult by conventional criteria, and the evaluation of hemodynamics is expected to become important in the assessment of its efficacy[16]. Recently, the modified RECIST (mRECIST)[17] and Choi criteria[18] have been recommended for the evaluation of therapeutic effects[19]. Also, in consideration of the serious complications of sorafenib reported to date[10], and moreover the fact that sorafenib is an expensive drug, early evaluation of its therapeutic effects is considered to be necessary to assess whether the treatment should be continued.

In this study, we performed image analysis by AtPI using CEUS with Sonazoid before and after the sorafenib administration and evaluated the usefulness of AtPI in evaluating early responses to sorafenib.

MATERIALS AND METHODS

Of the 45 patients with advanced HCC in whom treatment with sorafenib was initiated at our hospital between June 2009 and October 2011, 14 who consented to the study and were orally treated with sorafenib for at least 4 wk, and who could be followed up by CEUS, were selected as subjects. All patients were males with a mean age of 70.4 years (62-82 years). Underlying liver disease was hepatitis C in 8, alcoholic hepatitis in 4, and others in 2. Child-Pugh liver function class was A in all subjects, median alpha-fetoprotein level before administration was 150.9 ng/mL (7.1-22 516 ng/mL), and median protein induced by vitamin K absence-II level was 1781 mAU/mL (12-259 000 mAU/mL). The initial dose of sorafenib was 800 mg/d for 5 patients and 400 mg/d for 9 patients.

Methods

CEUS was performed before and 2 and 4 wk after the sorafenib administration. One lesion or portal vein tumor thrombus (PVTT) that could be followed for a period was selected on employing ultrasonography in each patient to standardize evaluations, and CEUS was performed in the same cross-section and under the same conditions at all time points. The ultrasound equipment used in this examination was SSA-790A (Toshiba Medical Systems, Tokyo, Japan) with a convex probe (PVT-375BT, 3.75-MHz center frequency). The imaging mode used was wideband harmonic imaging (pulse subtraction) with transmission/reception frequencies of 1.8 and 3.5 MHz, respectively. The mechanical index for acoustic output was set to 0.2; the dynamic range was set to 60-65 dB. A single focus point was set at the deep site of the lesion, and a bolus intravenous injection of Sonazoid (0.5 mL) was administered *via* a left cubital venous line followed by 10 mL normal saline flush. After injection of Sonazoid, the patients were asked to hold their breaths. The arterial phase (0-40 s) was observed and video images were recorded and analyzed by an off-line procedure using AtPI.

AtPI was performed using image analysis software for Aplio/Xario on the basis of the report by Watanabe *et al*[2]. It was performed by determining a starting point at an appropriate site such as an intrahepatic artery and a tumor vessel, regarding the time when the contrast agent reached this site as the zero point, measuring the difference in the arrival time between the target and starting points throughout the entire diagnostic image, and coloring the time differences [color mapping (CM)]. In this study, the moment of arrival of the contrast agent at a

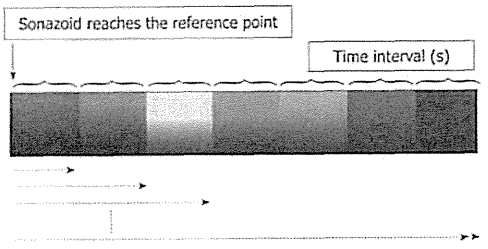

Figure 1 Delays in the arrival of the contrast agent at the target site compared with that at the reference point (0 s) are represented by red, orange, yellow, green, light blue, blue and dark blue at 0.5 s intervals.

large artery near the tumor or PVTT was regarded as starting point.

Qualitative analysis: Delays in the arrival of the contrast agent at the target site compared with that at the reference point (0 s) were represented by red→orange →yellow→green→light blue→blue→dark blue at 0.5 s intervals (Figure 1). CM images obtained were grossly compared in each patient before and after the treatment.

Quantitative analysis: In prepared CM images, a maximum region of interest (ROI) was determined for each subject, and the mean arrival time of the contrast agent in the ROI from the starting point [mean time (MT)] was calculated. In each patient, differences in the MT 2 and 4 wk after the initiation of the treatment compared with the MT before treatment were determined. Blood flow velocity was judged to have been reduced when the difference was zero or greater [MT (+) group] and to have been increased when the difference was less than zero [MT (-) group]. Differences in the MT 2 and 4 wk after the treatment were compared with responses evaluated 4-8 wk after the treatment by dynamic computed tomography (CT), and statistical analysis was performed. mRECIST[21] was used for the response evaluation. The Mann-Whitney test was performed for statistical analyses at the $P < 0.05$ level of significance. This study was approved by the Ethical Review Board of Toho University Medical Center, Omori Hospital.

RESULTS

In 14 patients, the mean duration of sorafenib administration was 177 d, and the mean daily dose was 542.9 mg/d.

Qualitative analysis

In CM images 2 wk after the treatment, color in the tumor changed to primarily blue or dark blue from primarily red or yellow before the treatment in 8 of the 14 patients, and time-dependent changes (delays in the arrival time of the contrast agent) were noted. These 8 patients also showed similar changes in CM images 4 wk after the treatment (Figure 2). In the other 6 patients, no color changes were observed in the tumor, or red and/or yellow increases, between images 2 wk after the treatment and those before the treatment. These 6 patients also showed similar CM images 4 wk after the treatment.

Quantitative analysis

The MT was (+) in 8 patients but (-) in 6 two weeks after the treatment, and mean differences in MT were 1.21 ± 0.75 s and -0.85 ± 0.78 s, respectively. Four weeks after the treatment, the MT was (+) in 7 and (-) in 7, and the mean difference in MT was 1.18 ± 0.4 s and -1.11 ± 0.62 s, respectively. The MT was (+) but changed to (-) from 2 to 4 wk after the treatment in 1 of the 8 patients in whom CM images showed gross delays in the arrival time of the contrast agent in the tumor 2 and 4 wk after the treatment.

Dynamic CT could be performed 4-8 wk after the treatment in 13 of the 14 patients. Median differences in the MT were 1.13 s and 1.015 s, 2 and 4 wk after the treatment, respectively, in the 8 patients who showed stable disease (SD)/partial response (PR) on dynamic CT. Median differences in the MT were -0.39 s and -0.95 s, 2 and 4 wk after the treatment, respectively, in the 5 patients who showed progressive disease (PD). Differences in the median MT between SD/PR and PD groups were significant 2 and 4 wk after the treatment with $P = 0.019$ and $P = 0.028$, respectively (Figure 3). The finding on dynamic CT after treatment was SD in the above patient in whom the MT changed from (+) to (-) 4 wk after the treatment.

DISCUSSION

Some studies have evaluated the responses of gastrointestinal stromal tumor (GIST) and metastatic lesions of renal cell carcinoma to molecular-targeted agents by CEUS using Levovist (Schering, Berlin, Germany) and SonoVue (Bracco, Milan, Italy)[20-24]. Lassau *et al*[20] evaluated the responses of GIST to imatinib by CEUS using Levovist and SonoVue, and suggested that CEUS was useful for detailed evaluation early after the treatment, and that decreases in tumor staining early after the treatment were related to progression-free survival (PFS) and may serve as a response-predicting factor. Lamuraglia *et al*[21] also evaluated the efficacy of sorafenib against liver metastases of renal cell carcinoma by CEUS using SonoVue, and suggested that PFS or overall survival (OS) may be prolonged by the suppression of vascularization.

In this study, we evaluated the early responses of advanced HCC to sorafenib by AtPI using CEUS with Sonazoid. Differences in the arrival time of the contrast agent between a large artery near the target lesion and ROI were evaluated according to CM images or the MT. Evaluations of differences in the arrival time are considered equivalent to evaluations of blood flow velocity to the tumor or inflow volume of blood flow in the tumor.

In this study, a delay in the arrival time of the contrast agent, i.e., a decrease in blood flow velocity, could be detected visually and readily in CM images as early as 2 wk after the treatment in 8 of the 14 patients. A decrease in blood flow velocity could also be confirmed

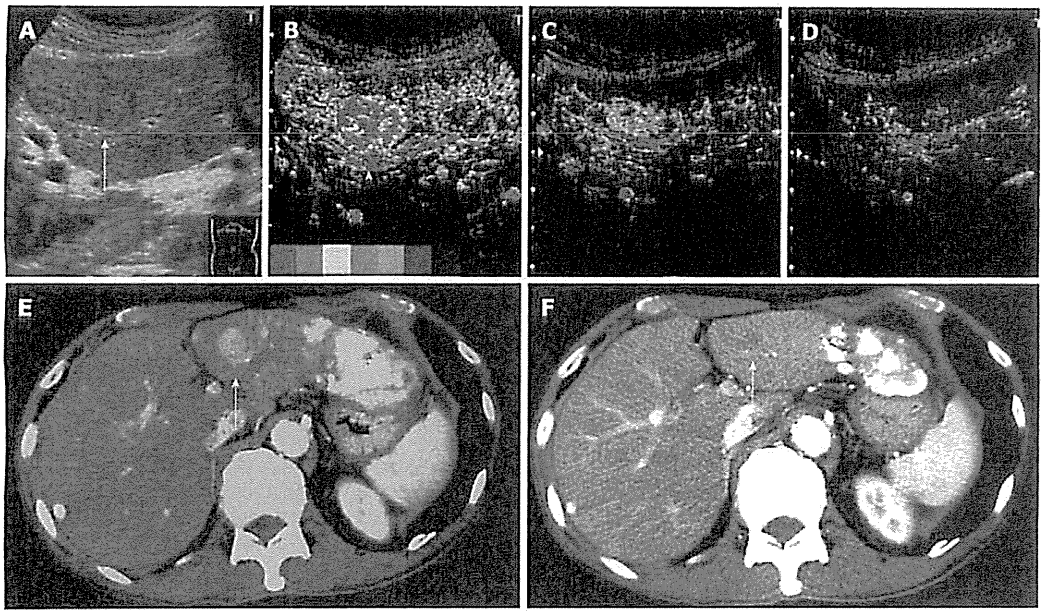

Figure 2 Clinical example of good responder patient. A 74-year-old man with a history of hepatitis C virus cirrhosis underwent transcatheter arterial chemoembolization (TACE) for advanced hepatocellular carcinoma three times, after which sorafenib administration (400 mg/d) was started for this patient because the tumor was TACE-refractory. A: Gray-scale ultrasonography showed a mosaic-pattern tumor sized 20 mm in diameter in S3 (arrow). This tumor was established as a target lesion; B: A large artery near this tumor was regarded as starting point (arrow head). The color mapping (CM) image before the treatment showed primarily red or orange in the tumor; C: The CM image 2 wk after the treatment showed primarily green in the tumor; D: The CM image 4 wk after the treatment showed primarily dark blue in the tumor, the same as the surrounding parenchyma; E: Dynamic computed tomography (CT) scan in arterial phase before the treatment showed a hypervascular lesion in S3 (arrow) which was the target lesion and accumulation of iodized oil in the left lobe and S7; F: Dynamic CT scan in arterial phase 4 wk after the treatment showed a hypovascular lesion in S3 (arrow) and this lesion reduced. This therapeutic response was described as a partial response.

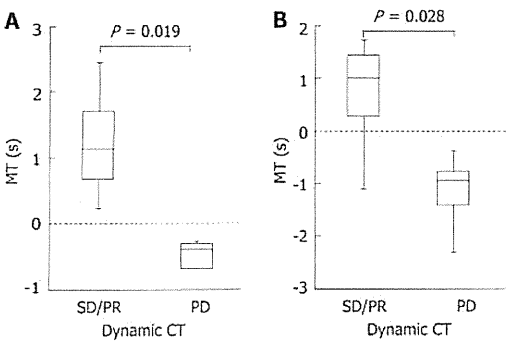

Figure 3 Comparisons between the mean time and therapeutic effects according to modified response evaluation criteria in solid tumors evaluated by dynamic computed tomography. A: Median difference in the mean time (MT) was 1.13 s, 2 wk after the treatment in the 8 patients who showed stable disease (SD)/partial response (PR) on dynamic computed tomography (CT). Median difference in the MT was -0.39 s, 2 wk after the treatment in the 5 patients who showed progressive disease (PD); B: Median difference in the MT was 1.015 s, 4 wk after the treatment, in the 8 patients who showed SD/PR on dynamic CT. Median difference in the MT was -0.95 s 4 wk after the treatment in the 5 patients who showed PD. Differences in the median MT between SD/PR and PD groups were significant 2 and 4 wk after the treatment with $P = 0.019$ and $P = 0.028$, respectively.

after 4 wk. However, as changes in blood flow in the tumor due to sorafenib administration were uneven, we set a ROI in the target HCC, calculated the mean arrival time of the contrast agent into the ROI, i.e., MT, and evaluated differences in the MT before and after the treatment as an objective method, not dependent on vision. In the 8 patients in whom delays in the arrival time of the contrast agent could be confirmed visually in CM images both 2 and 4 wk after the treatment, the MT was (+) in all 8 after 2 wk and in 7 after 4 wk. This suggests the possibility of quantification and objective evaluation of changes in blood flow velocity in the tumor visually detected in CM images. Comparisons between the MT and therapeutic effects according to mRECIST evaluated by dynamic CT showed significant differences in the MT between SD/PR and PD groups both 2 and 4 wk after the treatment, indicating general agreement between these parameters. The results of this study suggest that a delay in the arrival time of the contrast agent visually detected in CM images, i.e., a change of CM images, reflect the response and that changes in hemodynamics visually represented in CM images may be quantified and objectively evaluated using the MT.

Responses of HCC have been evaluated primarily using dynamic CT and magnetic resonance imaging as well as CEUS, but it has often been difficult to repeat the examination frequently during the course due to exposure, iodine allergies, and renal dysfunction. On the other hand, Sonazoid is effective at a low dose, causes few adverse reactions, and can be used safely even in patients with iodine allergies or renal dysfunction, with the

exception of those with egg shell allergies. Since changes in CM images were noted in this study, and the MT showed differences from values before the treatment by 2 wk after the treatment, AtPI using CEUS with Sonazoid may be useful for the early evaluation of responses to sorafenib.

Lassau *et al*[22] reported that evaluation of the time to peak intensity and slope of the wash-in obtained by TIC using CEUS with SonoVue was useful for the predication of early responses of metastatic lesions of renal cell carcinoma to molecular-targeted agents. These techniques including AtPI reflect tumor hemodynamics, and their relative usefulness is impossible to discuss, but AtPI may be equally predictive of responses compared to the TIC.

This study raises the following questions: (1) Since tumors have a three-dimensional structure, can they be evaluated accurately by examination of a single cross-sectional ultrasonography image? (2) Since HCC shows multicentric carcinogenesis[25,26] and intrahepatic metastasis, the degree of differentiation may vary among lesions of multiple HCC. Therefore, is it possible to apply the findings in a single target lesion uniformly to all other lesions? (3) Visual evaluation of CM images obtained 4 wk after the treatment disagreed with the MT value in 1 patient. While CM images were found to be useful for simple comparison between conditions before and after the treatment, measurement of the MT is still a complex process, and further improvements are considered necessary in the method to determine the ROI and other aspects; and (4) Do the results obtained by this study, i.e., the MT calculated by AtPI, contribute to improvements in the OS? These problems are considered to need careful evaluation by further accumulation of cases.

In conclusion, AtPI by CEUS using Sonazoid is suggested to be useful for evaluating early responses to sorafenib. Changes in hemodynamics in advanced HCC over the course of its treatment could be visually represented in a single static CM image obtained by AtPI. By further quantification, hemodynamic changes over the course of treatment could be evaluated more objectively. Since the number of patients in this study was small, and the observation period was short, the usefulness of the procedure in evaluating the time to progression or OS cannot be discussed, but evaluation of tumor hemodynamics using AtPI is considered to contribute to the prediction of responses early after treatment.

COMMENTS

Background

Recently, molecular biological characteristics related to the progression and proliferation of hepatocellular carcinoma (HCC) have been clarified, accelerating the development of various molecular-targeted agents. Since sorafenib is a multikinase inhibitor and it targets tumor growth and angiogenesis signal transduction pathways, it is expected to be useful for the treatment of HCC.

Research frontiers

Therapeutic efficacy has been assessed by Response Evaluation Criteria in Solid Tumor using dynamic computed tomography (CT) and overall survival in previous studies. Since sorafenib produces an antitumor effect primarily by inhibiting angiogenesis, evaluation of its antineoplastic effect is difficult by conventional criteria, and evaluating hemodynamics is expected to become important for its efficacy evaluation. In this study, the authors verified that arrival time parametric imaging (AtPI) by contrast-enhanced ultrasonography (CEUS) using Sonazoid is useful for evaluating early responses to sorafenib.

Innovations and breakthroughs

Dynamic CT and magnetic resonance imaging are generally used to evaluate the antineoplastic effect, but tumor hemodynamics are difficult to evaluate by these methods. AtPI by CEUS can evaluate the changes of hemodynamics in HCC over a course of treatment visually and objectively.

Applications

AtPI by CEUS using Sonazoid is suggested to be useful for evaluating early responses to sorafenib. This was a pilot study with a small series of patients. Similar studies with much larger numbers of patients are awaited.

Terminology

Ultrasound contrast agents consist of microbubbles that visualize the hemodynamics. AtPI is a method using CEUS, which utilizes the arrival time of each pixel in the diagnostic image.

Peer review

This is an interesting manuscript on a small series of patients followed with CEUS under treatment with sorafenib. As a pilot, it provides an interesting hypothesis to be tested in a larger series of patients. It is well conducted and also well presented.

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ORIGINAL RESEARCH

Clinical factors related to long-term administration of sorafenib in patients with hepatocellular carcinoma

This article was published in the following Dove Press journal:
Cancer Management and Research
17 December 2012
Number of times this article has been viewed

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Background: Sorafenib has been approved in the indication of unresectable hepatocellular carcinoma, but there are many cases in which administration of the drug is discontinued due to severe side effects. In this study, we compared the characteristics of patients who continued and discontinued sorafenib.

Methods: Ninety-six patients (75 men and 21 women) were initiated on sorafenib from July 2009 through September 2011. The patient characteristics of interest included gender, age, etiology, Child-Pugh classification, treatment history and frequency, and levels of α-fetoprotein, des-gamma-carboxy prothrombin, aspartate amino acid transferase, and alanine aminotransferase. Duration of administration of sorafenib and reasons for its discontinuation were compared.

Results: Median overall survival was 11.8 months. Discontinuation of sorafenib within 90 days was identified as an independent prognostic factor for overall survival on multivariate analysis ($P < 0.0001$). Transarterial chemoembolization performed six times or more ($P = 0.013$) was also identified as an independent factor contributing to discontinuation of sorafenib within 90 days in multivariate analysis. Patients who received sorafenib for ≥90 days had significantly longer overall survival than those who discontinued it ($P < 0.0001$).

Conclusion: Prolonged treatment with sorafenib is an important factor in achieving extended overall survival. We recommend starting sorafenib before latent liver damage has occurred as a result of too many transarterial chemoembolization procedures.

Keywords: sorafenib, hepatocellular continuation, discontinuation, efficacy

Introduction

In general, hepatocellular carcinoma in its early stages can be treated by surgical resection, radiofrequency ablation,[1,2] or liver transplantation when there is a single nodule ≤ 5 cm or three nodules ≤ 3 cm (Milan criteria).[3] However, many people are diagnosed in the advanced stages, when only transcatheter arterial infusion chemotherapy and transarterial chemoembolization are performed,[4,5] but despite recently improved embolization devices,[6] even these therapies have limited success in cases of vascular invasion or extrahepatic spread.[7] Because hepatocellular carcinoma has a high recurrence rate, it is important to prevent secondary disease, and several therapies have been reported to prevent recurrence, including interferon,[8] retinoids,[9] and branched-chain amino acids.[10]

Recently, a number of molecularly targeted agents have been investigated throughout the world,[11–13] and some agents are entering Phase II or III trials.[14,15] Sorafenib inhibits the serine/threonine kinases, RAF-1and B-Raf,[16,17] inhibits the tyrosine kinase activity of vascular endothelial growth factor receptors 1, 2, and 3 and

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http://dx.doi.org/10.2147/CMAR.S38684

platelet-derived growth factor receptor β,[16,17] which has been shown to trigger production of reactive oxygen species and death of hepatocellular carcinoma cells.[18]

Sorafenib was approved to treat hepatocellular carcinoma in 2007,[19] and is used in patients with advanced stage disease. In two well known studies, sorafenib significantly increased survival time in patients with hepatocellular carcinoma, although there were absolute differences in survival time between the two patient populations because the definition of advanced disease differed between the two studies.[20,21]

Although administration of 800 mg of sorafenib is recommended, there are many cases in which administration is discontinued or the dosage is reduced because of severe side effects.[15,20,22] In this study, we demonstrated the therapeutic effects of sorafenib retrospectively and compared the characteristics of patients who continued and discontinued treatment with this agent.

Materials and methods

Patients

We enrolled 96 patients who had started to receive the drug in our hospital or at one of six affiliated hospitals from July 2009 through September 2011 and were able to be observed for more than 90 days. The patient characteristics investigated included gender, age, etiology, Child-Pugh classification, treatment history and frequency, levels of α-fetoprotein, des-gamma-carboxy prothrombin, aspartate amino acid transferase, and alanine aminotransferase, as well as treatment received prior to administration of sorafenib, eg, surgery, percutaneous ethanol injection therapy, percutaneous microwave coagulation therapy, radiofrequency ablation, transarterial chemoembolization, transcatheter arterial embolization, and/or transcatheter arterial infusion. The effectiveness of the treatments was evaluated according to modified Response Evaluation Criteria In Solid Tumors (RECIST)[23] using an enhanced computed tomography scan every 3 months. Duration of administration of sorafenib was noted and the reasons for its discontinuation were identified.

Treatment plan and toxicity evaluation

Sorafenib was initiated at 800 mg/day in two divided doses,[24] and dose reduction was allowed for unacceptable adverse effects, ie, grade 3/4 toxicities, and treatment was continued until disease progression, development of intolerable drug toxicity, or patient refusal to continue taking the drug. Patients were followed up on an outpatient basis every 2–4 weeks. Adverse events were assessed according to the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0.

Statistical analysis

Overall survival was estimated using the Kaplan-Meier method, and differences in survival between the groups were compared using the log-rank test. Cox's proportional hazard model and logistic regression were used to examine likely prognostic factors in each group. The results were reported as hazard ratios with 95% confidence intervals. $P < 0.05$ was considered to be statistically significant for all analyses. All statistical analyses were performed using JMP version 7.2 software (SAS Institute, Cary, NC).

Results

Patient characteristics

The 96 patients comprised 75 men and 21 women of mean age of 71.2 ± 16.8 (median 71) years (see Table 1). The etiology was hepatitis C virus in 56, hepatitis B virus in 15, and others in 25. Median (range) levels of

Table 1 Baseline patient characteristics and previous therapy before administration of sorafenib

Variable	Median (range)
Age	71 (52–87)
Gender (M/F)	75/21
HCV/HBV/others	56/15/25
Aspartate transaminase (U/L)	54 (19–165)
Alanine aminotransferase (U/L)	33 (12–150)
Platelets (per μL)	12 (5–32)
α-fetoprotein (ng/mL)	88.7 (3.2–245,500)
des-gamma-carboxy prothrombin (mAU/mL)	559 (5–75,000)
Child-Pugh classification (5/6/7)	62/30/4
BCLC stage B/C	37/59
Extrahepatic spread (±)	27/69
Macroscopic vascular invasion (±)	30/66
ECOG performance status (0/1/2)	50/44/2
Previous therapy	**n (%)**
Operation	32 (33)
Percutaneous ethanol injection therapy	11 (11)
Percutaneous microwave coagulation therapy	1 (1)
Radiofrequency ablation	42 (44)
TA(C)E	72 (75)
Transcatheter arterial infusion	19 (20)
Radiation	5 (5)
Efficacy in all patients	
Level of response	**n (%)**
CR	0 (0)
PR	13 (14)
SD	31 (32)
PD	30 (31)
Not evaluable	22 (23)
Response rate	14%
Disease-control rate	46%

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCV, hepatitis C virus; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; TACE, transarterial chemoembolization.

α-fetoprotein, des-gamma-carboxy prothrombin, aspartate (U/L), and alanine aminotransferase (U/L) were 88.7 (3.2–245,500) ng/mL, 559 (5–75,000) mAU/mL, 54 (19–165) IU/L, and 33 (12–150) IU/L, respectively. Fifty, 44, and two patients had an Eastern Cooperative Oncology Group performance status of 0, 1, and 2, respectively. Child-Pugh classification was 5 in 62, 6 in 30, and 7 in four. Barcelona Clinic Liver Cancer (BCLC) staging was B in 37 and C in 59. Twenty-seven patients had metastatic disease in organs other than the liver. Prior medical history at the time of initiation of sorafenib therapy was surgery in 32, percutaneous ethanol injection therapy in 11, percutaneous microwave coagulation therapy in one, radiofrequency ablation in 42, transarterial chemoembolization and/or transcatheter arterial embolization in 72, transcatheter arterial infusion in 19, and radiation in five cases.

Efficacy, response, and disease control rates

Median overall survival was 11.8 months. No patients had a complete response, 13 patients (14%) had a partial response, and 31 (32%) had stable disease (according to modified RECIST criteria), whereas 30 patients (31%) had progressive disease and 22 patients were not evaluable. The disease control rate was 46% and the response rate was 14% (see Figure 1 and Table 1).

Compliance with treatment

The mean sorafenib dose was 800 mg in 26 patients (27%), 600 mg in nine (9%), 400 mg in 46 (48%), and ≤200 mg in 15 (16%). Because the median duration of treatment was 87 (range 2–737) days, we divided the patients into two groups, ie, one with ≥90 days of treatment with sorafenib (n = 45) and another within 90 days of treatment with sorafenib (n = 51), and examined factors influencing the patient's ability to take the drug on a longer-term basis (Table 2). Common reasons for discontinuation within 90 days were adverse events (40 patients) and radiologic progression (n = 11). The adverse events were hand-foot skin reactions (n = 3), diarrhea (n = 3), general fatigue (n = 2), rash (n = 3), fever (n = 3), renal failure (n = 2), pancreatitis (n = 1), liver dysfunction (n = 15), and others (n = 8), whereas severe liver dysfunction included liver failure (n = 7), hepatic encephalopathy (n = 3), ascites (n = 3), elevation of aspartate or alanine aminotransferase (n = 1), and jaundice (n = 1).

Prognostic factors for overall survival by univariate and multivariate analysis

On univariate analysis, BCLC (C) staging ($P = 0.04$), tumor volume ≥ 50% of the liver ($P < 0.0001$), macroscopic vascular invasion ($P = 0.006$), and discontinuation of sorafenib administration within 90 days ($P < 0.0001$) were significant prognostic factors, but only discontinuation of sorafenib within 90 days was identified as an independent prognostic factor contributing to overall survival on multivariate analysis ($P < 0.0001$, Table 3).

Relationship between administration for ≥90 days and overall survival

In the group that continued on sorafenib for ≥90 days, overall survival was significantly longer than in the group that discontinued sorafenib within 90 days ($P < 0.0001$), and the same relationship was found in the 61 patients who had their dose reduced to 400 mg and 200 mg ($P = 0.0026$,

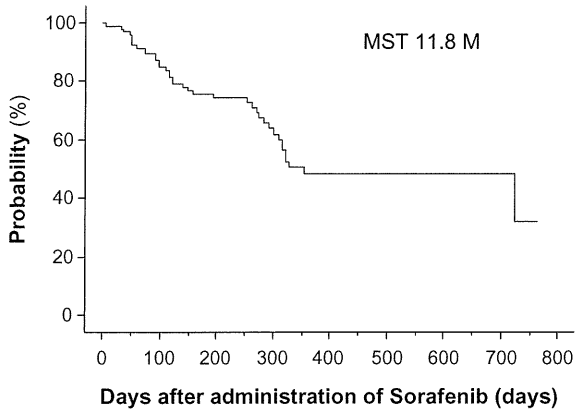

Figure 1 Overall survival for all patients.
Note: Median overall survival was 11.8 (range 7–763) days.

Table 2 Reasons for discontinuation of sorafenib within 90 days

Adverse events without liver dysfunction	**36**
Progressive disease	11
Hand-foot skin reaction	3
Diarrhea	3
General fatigue	2
Rash	3
Fever	3
Renal failure	2
Pancreatitis	1
Others	8
Liver dysfunction	**15**
Liver failure	7
Hepatic encephalopathy	3
Ascites	3
Elevation of AST or ALT	1
Jaundice	1

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase.

Table 3 Risk factors contributing to overall survival (n = 96)

	Subgroup	Univariate analysis			Multivariate analysis		
		Risk ratio	95% CI	*P* value	Risk ratio	95% CI	*P* value
Age	≥71 or <71	0.74	0.39–1.41	0.36			
Gender	Female	1.53	0.94–2.51	0.08			
Child-Pugh classification	≥6 or 5	0.54	0.28–1.04	0.06			
α-fetoprotein (ng/mL)	<50 or ≥50	1.95	0.94–4.0	0.71			
Des-gamma-carboxy prothrombin (mAU/mL)	<400 or ≥400	1.69	0.85–3.3	0.13			
Etiology	HCV, others	0.74	0.39–1.41	0.37			
PS	≥1 or 0	0.66	0.35–1.27	0.22			
BCLC	C or B	0.46	0.22–0.97	0.04	0.96	0.38–2.45	0.94
RFA	0 or ≥1	1.25	0.65–2.40	0.51			
TACE	<6 or ≥6	1.5	0.74–3.03	0.26			
Tumor volume of liver	<50% or ≥50%	4.41	2.24–8.69	<0.0001	1.70	0.77–3.74	0.19
Macroscopic vascular invasion	None or +	3.06	1.61–5.82	0.006	1.76	0.75–4.09	0.19
Discontinuation	<90 or ≥90	0.1	0.04–0.26	<0.0001	0.13	0.05–0.34	<0.0001
Extrahepatic spread	None or +	1.61	0.83–3.13	0.16			

Abbreviations: BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; CR, complete response; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

Figures 2 and 3). Therefore, we concluded that treatment with sorafenib for ≥90 days achieves better overall survival, even at a reduced dose.

Risk factors for discontinuation of sorafenib within 90 days by univariate and multivariate analysis

To identify risk factors for discontinuation of sorafenib within 90 days, patients whose observed periods were less than 90 days were eliminated. The total number was 82, with 45 being ≥90 days and 37 being <90 days (Table 4). On univariate analysis, des-gamma-carboxy prothrombin (≤400 mAU/mL, $P = 0.04$), tumor volume ≥ 50% of the liver ($P = 0.02$), macroscopic vascular invasion ($P = 0.03$), and six or more transarterial chemoembolizations ($P = 0.02$) were significant factors, and tumor volume ≥ 50% of the liver ($P = 0.04$) and six or more transarterial chemoembolizations ($P = 0.013$) were identified as independent risk factors on multivariate analysis.

Discussion

Sorafenib is considered a drug that should be used for advanced hepatocellular carcinoma, but there are no suitable criteria for treatment of hepatocellular carcinoma at this stage, because the treatment outcomes are affected by multiple variables, including liver function, patient performance status, and tumor stage.[25,26]

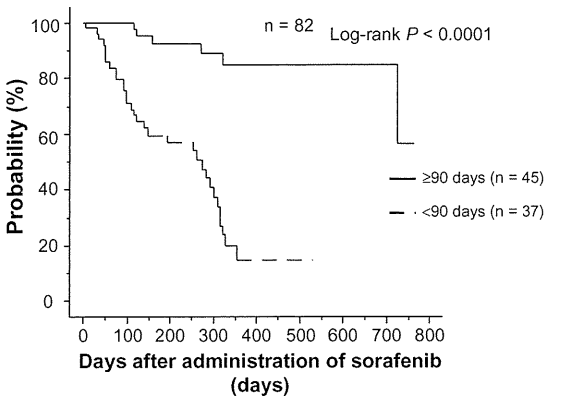

Figure 2 Relationship between continuation of administration and overall survival.
Note: In the group that continued administration for ≥90 days, overall survival was significantly higher than in the group that discontinued administration within 90 days.

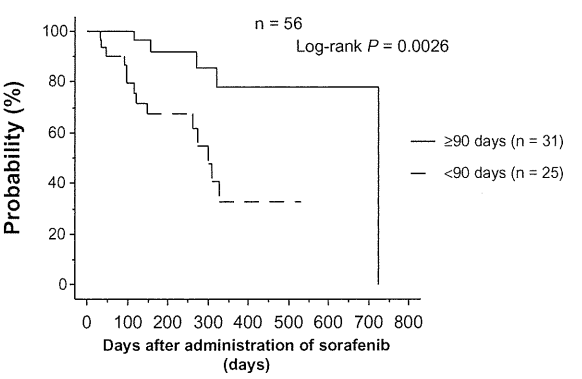

Figure 3 Relationship between continuation of administration and overall survival in patients receiving 200 mg or 400 mg.
Note: In the group that continued administration for ≥90 days, overall survival was significantly higher than in the group who discontinued administration within 90 days.

Table 4 Risk factors contributing to discontinuation of sorafenib administration within 90 days (n = 82)

	Subgroup	Univariate analysis			Multivariate analysis		
		Risk ratio	95% CI	*P* value	Risk ratio	95% CI	*P* value
Child-Pugh classification	≥6 or 5	2.10	0.83–5.30	0.11			
α-fetoprotein (ng/mL)	<50 or ≥50	0.55	0.22–1.35	0.19			
DCP (mAU/mL)	<400 or ≥400	0.39	0.16–0.97	0.04	0.59	0.22–1.60	0.30
Radiofrequency ablation	0 or ≥1	0.78	0.32–1.88	0.58			
Tumor volume of liver	<50% or ≥50%	0.08	0.01–0.69	0.02	0.99	0.01–0.91	0.04
Macroscopic vascular invasion	None or +	0.33	0.12–0.87	0.03	0.46	0.15–1.39	0.17
TACE	<6 or ≥6	0.26	0.08–0.83	0.02	0.21	0.06–0.72	0.013
Extrahepatic spread	None or +	1.81	0.67–4.92	0.24			

Abbreviations: CI, confidence interval; DCP, des-gamma-carboxy prothrombin; TACE, transarterial chemoembolization.

Many trials have reported on the use of sorafenib to prevent hepatocellular carcinoma recurrence after treatment.[27] Sorafenib is used with sirolimus or with inhibitors of mammalian target of rapamycin[29] to reduce the risk of recurrence of hepatocellular carcinoma after liver transplantation,[28] and it has been reported that such combination therapy can be effective. Some adjuvant therapy studies after curative treatment for hepatocellular carcinoma, such as the STORM (Sorafenib as adjuvant Treatment in the prevention Of Recurrence of hepatocellular carcinoMa) trial, are ongoing,[27] and concurrent treatment of hepatocellular carcinoma with conventional transarterial chemoembolization and sorafenib has demonstrated a longer time to progression and possible efficacy.[30,31] The overall median survival of our subjects was 11.8 months. Our data are slightly more robust than those of the Phase III SHARP (Sorafenib in Advanced Hepatocellular Carcinoma Assessment Randomized Protocol) trial (10.7 months)[20] and the tandem study in the Asia-Pacific region (6.5 months), probably because our study included a higher number of BCLC stage B patients than were included in the registration trials.[21]

Sorafenib has several side effects and is often discontinued when their grade becomes severe. Multikinase inhibitors such as sorafenib have unique clinicopathologic consequences, including hand-foot skin reactions and severe side effects.[32–34] The severity of hand-foot skin reactions is dose-related and depends on the duration, dosage, and accumulation of the drug.[35] Our data show that almost all patients had side effects, with about 40% of patients discontinuing sorafenib due to adverse events. However, some side effects may predict a response to sorafenib, such as early skin toxicity and diarrhea,[22,36–38] and methods have been reported for evaluating efficacy and overall survival.[39,40] No studies have reported the efficacy of treatment dose or duration because the Phase III trial for sorafenib used only 800 mg.[20] Okuwaki et al reported late-onset progressive disease, indicating that prolonged treatment with sorafenib may be beneficial.[41] In our data, several factors in univariate analysis, such as BCLC stage C, tumor volume ≥ 50% of the liver, macroscopic vascular invasion, and discontinuation of sorafenib within 90 days reduced overall survival, but discontinuation of sorafenib within 90 days was the only factor found to reduce overall survival in multivariate analysis. Patients able to take sorafenib for longer than 90 days had better overall survival than those who discontinued within 90 days, even if they could take only 400 mg or less than 400 mg because of side effects. Our data demonstrate the benefit of a long duration of treatment, even in cases of reduced dosage. Interestingly, upon further investigation of patients who could be observed for 90 days or more, multivariate analysis showed that a tumor volume occupying ≥50% of the liver and six or more transarterial chemoembolization procedures prior to initiation of sorafenib were significant prognostic indicators. Our data indicate that prolonged administration is an important factor in obtaining good overall survival, and is better started when the number of transarterial chemoembolizations is less than six. Many transarterial chemoembolization procedures can worsen liver function and, although the Child-Pugh score does not change, there might be latent liver damage.

Sorafenib is indicated for patients with BCLC stage C and transarterial chemoembolization is recommended for patients with BCLC stage B,[7,42] so sorafenib is usually used in patients whose tumors are progressing despite locoregional therapy. We recommend starting sorafenib before latent liver damage has occurred as a result of too many transarterial chemoembolization procedures, and prolonged administration of sorafenib is important for long overall survival, even if the dose of sorafenib needs to be reduced because of side effects.

Disclosure

The authors declare that they do not have anything to disclose regarding funding or conflicts of interest with respect to this manuscript.

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