Decreased Blood Flow after Sorafenib Administration Is an Imaging Biomarker to Predict Overall Survival in Patients with Advanced Hepatocellular Carcinoma

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Key Words
Arterial blood flow · Hepatocellular carcinoma · Overall survival · Sorafenib · Tumor staining

Abstract
Background: Sorafenib is a multikinase inhibitor targeting Raf and protein tyrosine kinases, which are involved in cell growth and tumor angiogenesis. Sorafenib administration induces temporary inhibition of tumor growth and a decrease in arterial blood flow in a considerable number of hepatocellular carcinoma (HCC) patients. We retrospectively evaluated the association between decreased blood flow and the overall survival (OS) of HCC patients after the initiation of sorafenib therapy. Patients and Methods: Therapeutic responses of 158 advanced HCC patients with hypervascular tumors who had received sorafenib for more than 1 month were analyzed. To assess their therapeutic response, patients underwent radiological evaluation before and every 4–6 weeks after the initiation of sorafenib treatment. After the classification of patients into three groups based on the change in arterial enhancement during treatment (no change, decrease and disappearance), the OS of each group was compared using the Kaplan-Meier method. Results: Statistically significant differences in OS were observed among the three groups (p < 0.001). A decrease or disappearance of arterial enhancement was significantly associated with improved OS compared to patients with no change in arterial enhancement; the median OS was 19.9 months (95% confidence interval, CI, 16.4–24.5 months) and 6.0 months (95% CI, 4.0–8.8 months), respectively (p < 0.001). However, there was no difference in OS between the decrease and disappearance groups (p = 0.88). Conclusion: We conclude that decreased arterial enhancement during sorafenib treatment was associated with the longest OS and could therefore reflect an effective response.

Introduction
Hepatocellular carcinoma (HCC) is currently ranked as the fifth most common cancer and the third leading cause of cancer-related death worldwide [1, 2]. Because
most patients are diagnosed at advanced stages, only about 30% of patients presenting with early-stage tumors undergo potentially curative therapies, such as surgical resection [3, 4], transplantation [5, 6] or percutaneous ablation [7–9]. In contrast, patients with unresectable HCC usually receive palliative treatments, such as transarterial chemoembolization (TACE) [10], radiotherapy [11, 12] or conventional chemotherapy [13], and some patients participate in clinical trials [10, 14–17]. Among these options, only TACE has been shown to lead to survival benefits [18, 19]. However, its application is often limited due to the presence of vascular invasion or extrahepatic spread [20–23].

Sorafenib, a small-molecule multikinase inhibitor [24], was the first systemic agent that was proven to prolong survival in patients with advanced HCC in two phase III trials [25, 26], and it is now the standard of care for systematically treated patients [13, 22, 27–30]. Sorafenib inhibits Raf protein kinase and receptor tyrosine kinases, including platelet-derived growth factor receptor and vascular endothelial growth factor receptor, which is involved in the neovascularization of HCC. In addition, sorafenib also inhibits Flt-3 and c-KIT, which are both involved in neovascularization and cellular growth [31–33]. However, because both trials were assessed using the Response Evaluation Criteria in Solid Tumors 1.1 (RECIST1.1), altered arterial enhancement and its association with overall survival (OS) in sorafenib-treated patients has not been evaluated thus far [34].

We reported that the modified RECIST (mRECIST) and the Response Evaluation Criteria in Cancer of the Liver (RECICL), which include the assessment of arterial tumor enhancement, are useful for evaluating therapeutic effects for HCC patients [35, 36]. Indeed, it has been reported that for patients treated with sorafenib, OS is better reflected by mRECIST than RECIST1.1 [37, 38], suggesting that a consequence of effective treatment may be the disappearance of tumor staining. Therefore, the association between decreased arterial enhancement and OS should be clarified.

In this study, we retrospectively examined the relationship between alterations in arterial enhancement determined by imaging and the survival of HCC patients who presented with hypervascular tumors and received sorafenib treatment. We demonstrated that patients with decreased tumor enhancement clearly demonstrated better OS than patients whose tumor staining remained unchanged. Therefore, a change in tumor enhancement should be a surrogate marker of tumor response after the initiation of sorafenib therapy.

Patients and Methods

Patients

Between May 2009 and November 2012, 269 patients with advanced HCC were treated with sorafenib at the Kinki University Hospital, and 158 patients who had received continuous sorafenib administration and met the inclusion criteria were selected for this retrospective study. Their response to sorafenib had been examined at least once using contrast-enhanced computed tomography (CE-CT) and/or dynamic magnetic resonance imaging (MRI).

The inclusion criteria for this study were: (1) a diagnosis of HCC based on histological examination or radiological findings showing early enhancement, followed by late washout on CE-CT or dynamic MRI in conjunction with HCC refractory to radiofrequency ablation and TACE based on the indications for sorafenib; (2) a performance status of 0 or 1, and (3) Child-Pugh class A or B. Exclusion criteria were: (1) concomitant antineoplastic treatment; (2) prior treatment with TACE or radiofrequency ablation less than 3 months before initiation of sorafenib treatment, and (3) lack of a response, which was assessed using CE-CT or dynamic MRI during the follow-up period.

Initial and Follow-Up Assessments

Liver function and tumor stage were evaluated using the Child-Pugh and Barcelona Clinic for Liver Cancer (BCLC) classifications. CE-CT images were obtained during the arterial (40 s) and portal (70 s) phases using 120 ml of iomeprol at a flow rate of 3 ml/s. Dynamic MRI scans were performed with gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) enhancement during the arterial (22–35 s after injection) and portal venous (70 s after injection) phases using a T1-weighted high-resolution sequence in a single breath hold. The CE-CT and Gd-EOB-DTPA-MRI scans were reviewed by two independent radiologists, and the size and arterial enhancement of the tumors were evaluated every 4–6 weeks during and after treatment. Every CT and Gd-EOB-DTPA-MRI scan was reviewed retrospectively by two independent hepatologists. Responses after initiation of treatment were evaluated separately according to mRECIST in a non-blinded fashion (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000368013) [35]. The target lesions were defined by both physicians for all patients on their CT and/or MRI scans before treatment. Extrahepatic lesions were assessed, as required, by chest radiography, bone scintigraphy or fluorodeoxyglucose positron emission tomography. Tumor markers were also measured every 4–6 weeks to assess tumor growth. OS analysis ended at the time of death or was censored at the time of the last follow-up visit. The criteria for sorafenib discontinuation were: (1) determination of progressive disease (PD) based on mRECIST, such as obvious tumor progression and/or onset of a new lesion; (2) grade 3 or greater adverse reactions that could not be controlled for by dose reduction or interruption based on the Common Terminology Criteria for Adverse Events, version 4.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf), and (3) noncompliance with oral drug treatment and/or follow-up visits.

Tumor Classification Based on Arterial Enhancement

The lesions analyzed in this study were a maximum of five lesions when more than five intrahepatic lesions were present. For the classification based on alterations in arterial enhancement, the
decrease group (DEC) was defined as patients showing a clear decrease in part or the entire area of tumor staining after the administration of sorafenib, while the disappearance group (DA) comprised patients showing disappearance of tumor staining within a part of the tumor area or a complete disappearance of tumor staining in all phases (i.e. the arterial, portal and late phases). The DEC group was defined as a >15% decrease in tumor density in the early phase using CT. The no change (NC) group did not meet the criteria of the DEC and DA groups regarding tumor density on a CT scan. The NC group comprised patients with neither a clear decrease nor a disappearance of tumor staining after sorafenib administration. We compared OS among these three groups. The response group consists of patients showing DEC or DA. We compared OS among the previously mentioned three groups, as well as between the response and NC groups.

**Statistical Analysis**

Univariate survival curves were estimated using the Kaplan-Meier method. Survival rates among groups were compared using the log-rank test, and categorical variables were compared using the χ² test. For multiple comparisons, the Bonferroni correction was applied. The level of significance was set at p < 0.05. All analyses were performed using SAS statistical software (version 8.2; SAS Institute, Cary, N.C., USA) or the SPSS Medical Pack for Windows (version 10.0; SPSS, Inc., Chicago, Ill., USA).

**Results**

**Characteristics of the Patients Enrolled in the Study**

The median OS of the entire cohort was 16.7 months (95% confidence interval, CI, 10.6–22.8 months). The numbers of patients with a complete response (CR), partial response (PR), stable disease (SD) and PD were 6, 30, 52 and 70, respectively. The response rate and disease control rate (DCR) estimated by mRECIST were 23.4 and 54.5%, respectively. Eighty-five patients (53.8%) were positive for anti-hepatitis C virus (HCV) antibody and were thus considered to have HCV-related HCC, while 26 patients (16.5%) had tested positive for hepatitis B virus.
rus (HBV) surface antigen and 47 patients (29.7%) had tested negative for both HCV antibody and HBV surface antigen. One hundred fifty-six patients were asymptomatic (performance status 0) and 69 patients (43.7%) were classified as BCLC stage A or B. One hundred twenty-eight patients (81.0%) were Child-Pugh class A.

Classification of Patients according to Alterations in Tumor Staining after the Initiation of Sorafenib

Sixty-one patients showed no change in tumor staining (NC group), while 97 patients showed a decrease in the entire area or part of the area of tumor staining. Of these 97 patients, 58 showed a complete disappearance of tumor staining in the arterial, portal and late phases (DA group), while 39 patients showed decreased tumor staining in terms of staining intensity compared to the staining before sorafenib administration in the arterial phase (DEC group). The characteristics of the patients in the three groups are summarized in table 1. The NC group consisted of 47 men and 14 women with a median age of 71 years; 51 were Child-Pugh class A and 10 Child-Pugh class B. From the NC group, 12, 30 and 19 patients had HBV-, HCV- and non-B, non-C (NBNC)-related HCC, respectively. The DEC group consisted of 26 men and 13 women with a median age of 73 years; 33 patients had liver cirrhosis of Child-Pugh class A and 6 patients were Child-Pugh grade B. From the DEC group, 6, 18 and 15 patients had HBV-, HCV- and NBNC-related HCC, respectively. The DC group consisted of 47 men and 11 women with a median age of 73 years; 44 patients were Child-Pugh class A and 14 patients Child-Pugh class B, while 8, 37 and 13 patients had HBV-, HCV- and NBNC-related HCC, respectively. With regard to patient characteristics, there were no statistically significant differences among the three groups. Among the patients analyzed, tumor responses were classified using the mRECIST system and were as follows: CR = 0, PR = 4, SD = 20 and PD = 37 patients in the NC group; CR = 2, PR = 7, SD = 18 and PD = 12 patients in the DEC group, and CR = 4, PR = 19, SD = 14 and PD = 21 patients in the DA group. According to RECICL, tumor response was classified as CR in 0, PR in 4, SD in 19 and PD in 38 patients in the NC group; as CR in 2, PR in 7, SD in 17 and PD in 13 patients in the DEC group, and as CR in 4, PR in 22, SD in 14 and PD in 18 patients in the DA group. When evaluated by mRECIST and RECICL systems, both the DEC and the DA group were associated with a higher objective response rate (ORR) and DCR compared to the NC group. Among the three groups, the highest ORR was observed in the DA group (table 2).

Relationship between Disappearance/Decrease of Tumor Staining and OS

The median OS was 6 months (95% CI, 4.0–8.8 months) in the NC group, 20.8 months (95% CI, 11.8–29.8 months) in the DEC group and 18.8 months (95% CI, 14.8–22.8 months) in the DA group. These differences between the groups were statistically significant. Patients of the NC group showed significantly shorter OS than patients of the DA group (p < 0.001) and the DEC group (p = 0.003). No difference in OS was detected between patients of the DEC and DA groups (p = 0.88; fig. 1a).

To further clarify the impact of altered enhancement during sorafenib treatment, we combined the DEC and DA groups as the response group and compared the OS of this group with that of the NC group. The response group comprised 73 men and 24 women with a median age of 73 years; 76 patients were Child-Pugh class A and 20 were Child-Pugh class B; 14, 55 and 28 patients had HBV-, HCV- and NBNC-related HCC, respectively. There were no statistically significant differences in patient characteristics between the NC group and the response group (online suppl. table 2). According to the mRECIST system, 6 patients had CR, 26 patients had PR, 32 patients had SD and 33 patients had PD in the response group. Both ORR and DCR were increased in the response group compared to the NC group when evaluated by mRECIST and RECICL. OS of the response group was significantly different from that of the NC group (p <
0.001), with a median OS of 19.9 months (95% CI, 16.4–23.5 months) in the response group and 20.8 months (95% CI, 11.8–29.8 months) in the NC group (fig. 1b).

**Relationship between Disappearance/Decrease of Tumor Staining and OS in Patients Evaluated as PD by mRECIST**

According to mRECIST, tumor response was classified as CR in 6 and PR in 30 (objective response, OR), SD in 52 and PD in 70 patients. We further classified the patients with PD into two subgroups: the response PD was defined as patients with PD but a clear decrease or disappearance of tumor enhancement after sorafenib administration, while the no-response PD group consisted of patients without any change in tumor enhancement. Comparisons of the survival curves showed that the median OS was 25.4 months (95% CI, 13.4–37.4 months) in the tumor response group, 20.8 months (95% CI, 12.7–28.9 months) in the SD group, 11.4 months (95% CI, 8.1–14.7 months) in the response PD group and 5.4 months (95% CI, 4.3–6.5 months) in the no-response PD group. The no-response PD group showed significantly shorter OS than both the SD group (p < 0.001) and the response PD group (p = 0.002). Although the response PD group showed shorter OS than the SD group, OS was not significantly different between the two groups (p = 0.050; fig. 2).

**Discussion**

In this study, patients who showed complete disappearance of tumor staining, which reflects necrosis of HCC lesions, showed better OS than the patients of the NC group. In addition, a better OS was also observed in the DEC group compared to the NC group, suggesting that a decreased arterial blood flow could be a partial effect of sorafenib. On the other hand, OS did not differ between DA and DEC groups. Based on the results of the present analyses, a decrease in arterial blood flow could lead to partial treatment effects in terms of OS compared to patients with disappearance of enhancement, where tumor necrosis was expected.
Currently, RECIST1.1, mRECIST and RECICL are mainly used to evaluate tumor response during treatment of HCC. RECIST1.1 are the simplest criteria where tumor necrosis is not taken into consideration and only the largest tumor diameter is assessed. However, as OS was better in patients showing a disappearance or decrease of tumor blood flow, alterations in tumor enhancement apparently reflect tumor response in HCC treatment. As our results suggest that decreased blood flow is of prognostic significance, it is conceivable to speculate that applying the assessment of tumor enhancement is more important than evaluating the decrease in tumor size during sorafenib treatment.

When treating advanced HCC patients with sorafenib, complete disappearance of tumor staining is considered to reflect tumor necrosis, while decreased tumor staining may represent the presence of variable tumors due to the presence of blood flow. While the disappearance or lack of disappearance of blood flow is included in the evaluation criteria in mRECIST and RECICL, decreased blood flow is not taken into consideration among these criteria. In this study, OS was longer in patients of the response group in terms of tumor enhancement. The decrease in blood flow could induce temporary inhibition of tumor growth, thereby suppressing HCC progression. In addition, among patients showing PD, those with response PD had better OS than those with no-response PD. From this point of view, patients with response PD should not stop sorafenib treatment even if they were regarded as PD according to the conventional criteria.

In this study, we also encountered patients who showed decreased tumor staining with administration of sorafenib, but still showed tumor enlargement. Although improvement in OS was observed in the group showing a decrease or disappearance of tumor enhancement, some patients with decreased tumor blood flow still showed a short survival (<6 months). It is also known that poorly differentiated HCC can sometimes represent the decrease in arterial blood flow that reflects arterial abnormalities [39]. Because the decrease in tumor blood flow is sometimes associated with malignant transformation, the clinical course must be carefully followed even if patients display disappearance or decrease of enhancement during treatment.

This study has two major limitations that may reduce the generalizability of our results to other populations with advanced HCC. First, because the determination of tumor enhancement is not quantitative, it might reflect interphysician differences in terms of image interpretations. The measurement of tumor enhancement might also be affected by the timing of the imaging. Second, due to the retrospective nature of the study, there could be a bias regarding patient selection. To address these limitations and independently validate the results of this investigation, we are currently designing a prospective multicenter study in a larger patient cohort.

In conclusion, even if tumor blood flow has not completely disappeared, prolonged survival of patients with advanced HCC can be expected with sorafenib treatment if the tumor blood flow decreases, even when tumor necrosis is not achieved. This result should be of importance for the management of HCC patients receiving sorafenib treatment.
References


